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Direktorin: Professor Dr. med. Antonia Joussen

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Dr. med. Milena Jasmin Elisabeth Pahlitzsch

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Dekan:	Prof. Dr. med. Axel R. Pries
1. Gutachter/in:	Frau Prof. Dr. Esther Hoffmann, Mainz
2. Gutachter/in:	Herr Prof. Dr. Gerd Geerling, Düsseldorf

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Abkürzungsverzeichnis

Aβ	β-Amyloid
CCT	central cornea thickness, zentrale korneale Dicke
CoQ10	Coenzym Q10
DARC	Detektion apoptotischer retinaler Ganglienzellen
IL	Interleukin
IOD	Intraokularer Druck
MIGS	Minimal-invasive Glaukomchirurgie
PEX	Pseudoexfoliationssyndrom
PEX-Glaukom	Pseudoexfoliationsglaukom
POAG/POWG	Primary open angle glaucoma/ Primäres Offenwinkelglaukom
QOL	Quality of life, Lebensqualität
EWG	Engwinkelglaukom
IOL	Intraokularlinse
OCT	Optical Coherence Tomography, Optische Kohärenztomographie
OHT	Okuläre Hypertension
PERG	pattern electroretinogram, Muster-Elektroretinogramm
RGC	Retinal ganglion cell, Retinale Ganglionzelle
TE	Trabekulektomie
Trabectome	Trabekulotomie ab interno
VEP	visuell evozierte Potenziale
z.B.	zum Beispiel

1. Einleitung

Das Glaukom ist definiert als eine progressive Optikusneuropathie, die durch Nervenfaserverluste zu fortschreitenden Gesichtsfelddefekten und irreversibler Blindheit führen kann[1,2]. Die pathophysiologischen Grundlagen der Erkrankung sind auch heutzutage nicht vollständig geklärt. Die Apoptose retinaler Ganglienzellen (RGC) spielt eine fundamentale Rolle in der Pathogenese [3,4]. Dem Soma-Zelltod geht häufig eine ausgeprägte axonale Degeneration der retinalen Ganglionzelle voraus[5–7]. Die Auslösemechanismen dagegen sind aktuell nicht eindeutig definiert; verschiedene Stoffwechselwege wurden identifiziert, die die Apoptose der RGCs einleiten oder unterstützen können[7]. Hervorzuheben sind die Glutamat-Exzitoxizität, Gliazell-Aktivierung, mitochondriale Dysfunktion und Autophagozytose. Eine Hochregulierung dieser Prozesse wird nicht nur in Glaukommodellen beobachtet, sondern auch in verwandten neurodegenerativen Erkrankungen wie Morbus Alzheimer und Morbus Parkinson[7–10].

Das Glaukom wird anhand der morphologischen intraokularen Strukturen in zwei große Bereiche unterteilt - das primäre Offenwinkelglaukom (POWG) und das Engwinkelglaukom (EWG). Auf das EWG wird in dieser Arbeit nicht näher eingegangen.

Das primäre Glaukom wird definiert als das Vorhandensein einer Optikusneuropathie mit einem normalen oder erhöhten intraokularen Druck (IOD) ohne Vorliegen einer ersichtlichen, sekundären pathologischen Ursache. Als sekundäre Glaukome werden Glaukomtypen mit einem erhöhten IOD und einem erkennbaren Grund dieser Druckerhöhung charakterisiert, beispielsweise Trauma, Inflammation, Pseudoexfoliationssyndrom (PEX), Pigmentdispersion und Neovaskularisationen[2]. Sekundäre Glaukomarten wie das Pseudoexfoliationsglaukom (PEX-Glaukom), Pigmentdispersionsglaukom und uveitische Glaukom spielen eine bedeutende Rolle im klinischen Alltag und werden anteilig in diese Arbeit eingeschlossen.

Der wichtigste uns bekannte Risikofaktor der Entstehung und Progression eines Glaukoms ist der IOD[11]. Auch heutzutage ist der IOD die Einflussgröße, die durch vorhandene Therapiemöglichkeiten am einfachsten zu modifizieren ist. Weitere Risikofaktoren sind Alter, Geschlecht, Abstammung und Wohnort (Land oder Stadt)[12–14]. Auch ein Flammer Phänotyp und vaskuläre Pathologien wie eine Migräne, Mikro- und Makroangiopathien im Rahmen eines Diabetes oder Nephropathie, Raynaud Phänomen und Schwankungen des arteriellen Blutdrucks stellen prädisponierende Faktoren für das Auftreten und die Progression einer glaukomatösen Neurodegeneration dar[11,15,16].

Diagnostische Möglichkeiten in der Erkennung eines glaukomatösen Schadens und Bestimmung der Progressionsrate liegen neben der klinischen Funduskopie und Gonioskopie in der Augeninnendruckmessung (nach Goldmann[17]), in der Perimetrie und in den bildgebenden retinalen Verfahren.

Die bildgebenden Verfahren haben in den letzten Jahren eine deutliche Weiterentwicklung erfahren, so dass sie heute standardmäßig zusätzlich zu der IOD Bestimmung und Perimetrie in der Glaukomdiagnostik als Erstuntersuchung und Verlaufskontrolle eingesetzt werden. Die wichtigsten Verfahren stellen die optische Kohärenztomographie (OCT), die Heidelberg Retina Tomographie (HRT) und die Farbfundusphotographie dar. Das Auge bietet die einzigartige Situation, das zentrale Nervensystem nicht-invasiv in vivo mittels bildgebender retinaler Verfahren darzustellen und strukturelle Veränderungen zu objektivieren. Auf die unterschiedlichen diagnostischen Verfahren wird in dieser Arbeit nicht weiter eingegangen.

Die aktuellen therapeutischen Möglichkeiten in der Behandlung des adulten POWG und Sekundärglaukoms lassen sich in zwei große Bereiche unterteilen; erstens die medikamentöse Therapie und operative Verfahren mit dem primären Ziel der intraokularen Drucksenkung bei individuell erhöhtem IOD und zweitens die Applikation neuroprotektiver Wirkstoffe als kausaler Therapieansatz eines neurodegenerativen Schadens.

Moderne Operationsmethoden in der Glaukomchirurgie werden vor allem so konzipiert, dass es eine Shunt Möglichkeit zwischen der Vorderkammer und dem Schlemm'schen Kanal gibt. Diese Verbindung führt zu einer Reduktion des juxtatrabekulären Widerstands, einer der wichtigsten anatomischen Lokalisationen einer Abflussstörung im Glaukom. Neben dem iStent® (1. Generation) und iStent inject® (2. Generation) hat sich die ab interno Kanaloplastik und vor allem die Trabectome® Chirurgie etabliert. Die minimal-invasive Glaukomchirurgie (MIGS) stellt eine sehr gute Alternative für die Bedürfnisse eines berufstätigen, modernen Patienten und auch eines Menschen höheren Alters mit anderweitig gelagerten Gesundheitsproblemen dar, der häufige Augenarztbesuche, tägliche medikamentöse Therapie mit begleitenden Nebenwirkungen und aufwendige Operationen nicht wahrnehmen kann und möchte. Die Verfahren Trabekulotomie ab interno (Trabectome®) und die iStent inject® Implantation führen zu einer effizienten IOD Reduktion und Senkung der Glaukomtherapeutika in dem primären Offenwinkelglaukom und den sekundären Glaukomtypen bei vorliegendem PEX- und Pigmentdispersionssyndrom[18–22]. Auch Kohorten zu uveitischen Sekundärglaukomen zeigen eine zuverlässige Reduktion des IOD mittels MIGS[23,24]. Weiter zählt die Goniotomie zu den minimal-invasiven Verfahren in der Glaukomchirurgie.

Der zweite Ansatz zur Therapie eines glaukomatösen Optikusschadens, der in dieser Arbeit diskutiert wird, ist die Wirkungsweise und Einsatzmöglichkeit neuroprotektiver Medikamente. Der Grundgedanke, neuroinflammatorische Prozesse kausal zu therapieren, ist aus dem Fachgebiet der Neurologie bekannt. Die Möglichkeit, neuroprotektive Wirkstoffe als etablierte Therapie standardisiert einzusetzen, steht dagegen noch am Anfang. Erste Medikamente sind zugelassen (Visufarma COQUN® Berlin, Germany) oder als alternative Heilmittel nicht rezeptpflichtig erhältlich. Die Herkunft neuroprotektiver Wirkstoffe findet sich vor allem in alten kulturellen Medizinrezepten, regionaler Küche und Pflanzen – heimisch als auch exotisch. Die Darreichungsform ist überwiegend oral oder als Tropfenapplikation möglich. In dieser Arbeit heben wir die Wirkung von Curcumin und Coenzym Q10 (CoQ10) als neuroprotektive Therapeutika hervor.

1.1. Glaukomchirurgie

Die Glaukomchirurgie lässt sich in zwei große Felder aufteilen: Filtrierende und nicht-filtrierende operative Techniken. Der filtrierende Mechanismus basiert auf der Eröffnung der Sklera mit Anlegen eines neuen Abflussweges aus der Vorderkammer durch die eröffnete Sklera unter die Bindehaut mit Aufnahme des Kammerwassers in den venösen Rücklauf.
Der Goldstandard der filtrierenden Verfahren ist die Trabekulektomie (TE). Diese wird unter 1.1.4. näher erläutert. Weitere Verfahren, auf die in dieser Arbeit nicht eingegangen wird, stellen die epibulbären Drainageimplantate (z.B. Ahmed valve, Baerveldt) und die Goniotrepanation dar.
Nicht-filtrierende Operationstechniken bilden die Grundlage der minimal-invasiven Glaukomchirurgie. Es erfolgt keine Sklera- Eröffnung und es wird konjunktivaschonend operiert. Häufig erfolgt ein ab interno Zugang durch eine clear cornea Inzision.
Neben der großen Unterteilung der filtrierenden und nicht-filtrierenden Glaukomoperationen gibt es verschiedene Ansätze der MIGS, die folgend ausgeführt werden. In dieser Arbeit wird eine spezielle Betonung auf das Trabectome® und iStent inject® Verfahren gelegt. XEN® Shunt Implantation, Kanaloplastik ab interno und das Cypass® Verfahren werden nicht tiefer erläutert.

1.1.1. Trabekulotomie ab interno (Trabectome®)

Die ab interno Trabekulotomie (Trabectome®, NeoMedix, Tustin, CA, USA) bietet eine mikro-invasive Alternative zu der penetrierenden Glaukomchirurgie mit dem Goldstandard

„Trabekulektomie“ für die operative Therapie von primären und sekundären Offenwinkelglaukomen[25–27]. Die Trabectome® Operation stellt eine nicht-filtrierende Möglichkeit ohne transsklerale Drainage zur Behandlung eines individuell erhöhten IOD dar. Entwickler dieser Methode sind George Baerveldt und Roy Chuck von der Irvine Universität, Kalifornien[28]. Die Technik ist seit 2009 in der EU für die Behandlung von Glaukompatienten verfügbar[29]. Eine milde bis moderate Schädigung des Nervus Optikus bei medikamentös nicht einstellbarem IOD oder Compliance Problemen wird als Indikation zur Operation gewertet[29].
Nach Aufsetzen einer Gonioskopie-Linse zum Einstellen des Kammerwinkels wird eine temporale clear cornea Inzision angelegt. Darauf wird das Handstück unter viskoelastischem Schutz in den nasalen Kammerwinkel eingeführt. Das Handstück verfügt über die Optionen Irrigation, Aspiration und Elektroablation. Über 120° wird das Gewebe des Trabekelmaschenwerks durch Elektroablation abgetragen und das Gewebe aspiriert.
Vorteile dieser Methode sind in der Gewebeentfernung durch Elektroablation und der technisch kontrollierbaren Eindringtiefe zu sehen[29].
Klinische Vergleichsstudien konnten einen langfristig drucksenkenden Effekt auf Werte um die 15 mmHg zeigen[20,30–33]. Weiter zeigten sich auch in der Kombination mit der Katarakt – Chirurgie stabile Ergebnisse in der IOD Reduktion[22,29,34].
Ergänzend konnten keine der filtrierenden Glaukomchirurgie ähnlichen Komplikationen wie eine persistierende Hypotonie, Blebitis, Endophthalmitis, expulsive Blutung und Vernarbung mit dem Trabectome® nachgewiesen werden[23,35,36].

1.1.2. iStent inject® Implantation

Der iStent inject® (GTS400, Glaukos, Laguna Hills, CA, USA) wurde als mikro-invasives Glaukomverfahren entwickelt. Es handelt sich um einen heparinbeschichteten Titan-Bypass mit einer Länge von 360µm und einem Durchmesser von 230µm, welcher CE-zertifiziert ist.
Das Funktionsprinzip beruht auf einer Ausbildung eines Umgehungskreislaufs des Trabekelmaschenwerkes zur Verbesserung des Kammerwasserabflusses. Die Implantation eines iStents® (1. Generation) führt in Vergleichsstudien zu einer effektiven Drucksenkung zwischen 16% und 33%, hauptsächlich in Offenwinkelglaukompatienten untersucht[21,37,38]. Der iStent inject® als weiterentwickelte Variante kann diese IOD Reduktion nochmals verbessern[39–41]. Neben einer fortlaufenden Designoptimierung und Größe des Bypasses werden in dem iStent inject® Verfahren standardmäßig zwei Stents eingesetzt[39–41]. Der Injektor ist mit zwei Stents

vorgeladen, die nach Anlage einer temporalen clear cornea Inzision unter gonioskopischer Sicht unmittelbar nacheinander im Abstand von 30-45° durch das nasale Trabekelmaschenwerk Richtung Schlemmkanal implantiert werden.
Das Ausmaß der Glaukomerkrankung, die Progressionsrate und der davon abhängige, individuelle Zieldruck spielen eine grundlegende Rolle bei der Patientenselektion. Die Zielgruppe mikro-invasiver Glaukomverfahren setzt sich vor allem aus Patienten mit einem milden und mittelschweren Glaukom und einem Zieldruck zwischen 15-21 mmHg zusammen[18,41–43]. Eine zuverlässige Drucksenkung kann in einem primären Offenwinkelglaukom, einem Pseudoexfoliationsglaukom oder einer okulären Hypertension (OHT) mit hohem Konversionsrisiko erreicht werden[39,41,42]. Das iStent inject® Verfahren kann singulär oder kombiniert im Rahmen einer Katarakt-Operation geplant werden[39,41,42].

1.1.3. Modifizierte Goniotomie

Die Goniotomie wurde von Otto Barkan 1948 zur Behandlung des kongenitalen Glaukoms entwickelt; nach wiederholter Behandlung kann eine Erfolgsrate von 80% im kindlichen Glaukom berichtet werden[44]. Es handelt sich um eine ab interno Prozedur mit Reduktion des trabekulären Abflusswiderstands und zusätzlichem Entfernen einer embryonalen Membran[44].
In ihrer Originalversion kann dieser großartige Erfolg am Erwachsenen nicht erreicht werden, so dass verschiedene Modifikationen entwickelt wurden. Nachfolgend der Ablauf unserer Operation[45]:
Die modifizierte Goniotomieoperation wird unter örtlicher Betäubung (peribulbär) in Miosis durchgeführt. Die Goniolinse (Hoskin-Barkan-Linse, Ocular Instruments, USA) wird auf die Hornhaut aufgesetzt, um den Kammerwinkel darzustellen. Anschließend wird das Trabekelmaschenwerk 120 Grad mit der Goniotomienadel eingeschnitten (Geuder, Deutschland). Zusätzlich wird eine Zyklodialyse (5 Uhr) angelegt (Teilschritt der Modifikation). Der Ziliarkörper wird vom Sklerasporn abgetrennt. Der suprachoroidale Raum wird durch sanfte Injektion des Viskoelastikums erweitert.
Alternativ gibt es auch eine Variante der Goniotomie mittels des Kahook Messers, auf die in dieser Arbeit nicht näher eingegangen wird[46].
Die modifizierte Goniotomie zeigt eine zuverlässige und effektive Drucksenkung auf Werte um 14 mmHG im 1-Jahresverlauf[45]. Weitere Vergleichsstudien dieser Modifikation mit anderen MIGS Verfahren sind unserem Wissen nach nicht publiziert.

Geeignete Patienten stellen sich vor allem mit einem milden bis moderaten POWG oder PEX-Glaukom vor[45]. Aufklärung über die Vielfalt verschiedener MIGS Verfahren mit Vor- und Nachteilen der jeweiligen Operation (z.B. Fremdkörper intraokular, Schnitt mechanisch oder Elektroablation) muss präoperativ erfolgen, um die richtige Patientenselektion sicher zu stellen.

1.1.4. Trabekulektomie

In diesem Kapitel wird eine der wichtigsten Glaukom-Operationen, die Trabekulektomie, als filtrierender Eingriff mit Senkung des IODs auf Werte <13mmHG ohne Lokaltherapie beschrieben:

Die penetrierende TE ist ein modifiziertes Verfahren der Goniotrepanation. Durch Anlegen eines Skleraflaps nach J. Fronimopoulos wird eine transsklerale Drainage aus der Vorderkanmer zu einer fortlaufenden IOD Reduktion geschaffen[47,48]. Das vorangehende Verfahren, deren Weiterentwicklung die TE darstellt, ist die Goniopunktion nach Elliot in den 30er Jahren[49]. Der Skleralappen ist das neu eingeführte Alleinstellungsmerkmal der TE und vermindert einen schnellen Kammerwasserverlust mit perioperativer Hypotonie. Das Operationsverfahren wurde in den 90er Jahren um die Anwendung von 5-Fluorouracil und Mitomycin C ergänzt, um die Komplikation einer postoperativen Narbenbildung signifikant zu reduzieren[50]. Klinische Studien konnten den Erfolg der TE vor allem in der Langzeitstabilität der Perimetrie und Papillenmorphologie ohne glaukomatöse Therapie bestätigen[51,52]. Weiter konnte der zentrale Visus langfristig aufrechterhalten werden[51–53]. Nachteile des Verfahrens sind expulsive Blutungen, Vernarbungen von Kornea, Konjunktiva und Sklera, Endophthalmitis, persistierende Hypotonie und Blebitis[26,54,55]. Zusammenfassend muss betont werden, dass es trotz seltener schwerwiegender Komplikationen wenig vergleichbare Operationstechniken gibt (XEN® als neu eingeführte Alternative), die eine IOD Reduktion zwischen 8 und 12 mmHg langfristig aufrecht erhalten können[53].

Das Verfahren der TE wird dargestellt, um Unterschiede zwischen penetrierender Chirurgie und MIGS hervorzuheben.

1.2. Neuroprotektive Wirkstoffe als kausaler Therapieansatz

Das Glaukom wird definiert als eine progressive irreversible Optikusneuropathie[1,2]. Als wichtigster Pathomechanismus wird die Apoptose retinaler Ganglienzellen als früheste Veränderung angenommen[4,7,56–59]. Bisher konnte nur der wichtigste uns bekannte Risikofaktor, der IOD, eingestellt werden, so dass diese Forschungsergebnisse einen Paradigmenwechsel einleiten. Der aktuelle Forschungsstand setzt sich kausal mit den Pathomechanismen des Auftretens eines glaukomatösen Schadens auseinander, die den programmierten Zelltod einleiten. Weiter sind hierfür moderne bildgebende diagnostische Verfahren entscheidend, die uns in vivo die Apoptose der retinalen Ganglienzellen darstellen und zeitlich nachverfolgen lassen.

Die multifaktorielle Genese des Glaukoms als neurodegenerative Erkrankung erklärt die Limitationen der medikamentösen und chirurgischen drucksenkenden Therapie; eine Progression ist auch bei optimal eingestelltem IOD über die Jahre zu beobachten[60]. Ein alternativer Ansatz zur Therapie der chronischen Inflammation, Aktivierung der Gliazellen und der Glutamat-Exzitoxizität ist notwendig, um nur einige histochemische Vorgänge zu der Entstehung und Progression des Glaukoms sowie anderer neurodegenerativer Erkrankungen zu nennen.

Neuroprotektive Wirkstoffe stellen eine vielseitige Gruppe unterschiedlichster Therapeutika dar, die hauptsächlich durch die Indische und Asiatische Küche und regionale Medizinrezepte nach Europa getragen wurden. Als wichtige Komponenten der aktuellen Forschung sind die Wirkstoffe Resveratrol, Curcumin, Ginsenoide, Grüntee Polyphenole und CoQ10 hervorzuheben[61–66]. Neuroprotektive topische und systemische Wirkstoffe kommen vor allem aus dem pflanzlichen Bereich. Wichtige Vertreter stellen pflanzliche Polyphenole dar, worunter auch das Curcumin und Resveratrol fällt. Beispielsweise ist eine heilende Wirkung des Curcumins in der Ayurveda Medizin seit 3000 Jahren bekannt.

Es muss betont werden, dass neuroprotektive Wirkstoffe nicht direkt kausal durch einen Pathomechanismus interagieren. Sie stellen vielmehr eine supportive Strategie dar und wirken durch multifaktorielle Kaskaden protektiv auf das neuronale Überleben. Der Wirkmechanismus neuroprotektiver Faktoren wird durch einen Anstieg der anti-oxidativen Aktivität, Reduktion apototischer Signale, Inhibition der Gliazellaktivität (insbesondere Mikroglia) und Herabsetzung pro-inflammatorischer Zytokine erklärt[61–66]. Neuroprotektive Medikamente lassen den IOD unbeeinflusst[67,68].

Ein weiterer Baustein in der Entstehung neurodegenerativer Erkrankungen ist das Vorhandensein einer mitochondrialen Dysfunktion. Der Verlust von CoQ10 führt zu einer Dysorganisation dieser überlebensnotwendigen Organelle, der zuerst physiologisch altersabhängig, aber auch in pathologischem Ausmaß in neurodegenerativen Erkrankungen wie Morbus Alzheimer, Morbus Parkinson und dem Glaukom auftritt[5,69].
In dieser Arbeit wird nachfolgend die Wirkung von Curcumin und CoQ10 erklärt und diskutiert. Andere Neuroprotektiva wie beispielsweise Citicolin oder Resveratrol sind nicht Gegenstand dieser Zusammenfassung.

1.2.1. Die Rolle des Polyphenols Curcumin

Curcumin (Diferuloylmethan) ist ein gelbes Pulver, gewonnen aus der Wurzel der Curcuma longa, zur Zingiberaceae Familie gehörend. Die Wirkkonzentration von Curcumin in einem indischen Curry liegt bei 2–5%.
Curcumin besitzt verschiedene biochemische Eigenschaften, die es als neuroprotektiven Wirkstoff klassifiziert: Es ist anti-inflammatorisch, anti-oxidativ und verhindert eine überschießende Protein-Aggregation[62–66].
Durch das Molekül werden pro-inflammatorische Signalkaskaden herunterreguliert, beispielsweise IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), Zyklooxygenase-2 (COX-2), Prostaglandin E-2 (PGE-2), die Interleukine-1–6–8 (IL-1, IL-6, IL-8) und der Tumornekrosefaktor-α (TNF-α)[62–66].
Weiter konnten anti-oxidative Eigenschaften des Heilmittels Curcumin in Ratten-Mikroglia durch H_2O_2-induzierte Zelltoxizität nachgewiesen werden[70]. Curcumin führt zu einem Anstieg des Zellüberlebens in Mikroglia und damit reduzierter Apoptoserate, und signifikanter Abnahme der freien Sauerstoffradikale, indem die Caspase 3, Cytochrom C und BAX enthaltenen Signalkaskaden heruntergefahren und BCL2 (protein B-cell lymphoma 2) hochreguliert wurde[70].
Die neuroprotektiven Charakteristika des Wirkstoffes Curcumin wurden sowohl in experimentellen Studien als auch in vivo in Tiermodellen und am Menschen in neurodegenerativen Erkrankungen, hauptsächlich in Morbus Alzheimer, Morbus Parkinson, Morbus Huntington, Glaukom, Trauma und Apoplex, analysiert und bestätigt[63,71–73].
Zusätzlich kann Curcumin β-Amyloid (Aβ) Plaques binden, die toxische Aβ Oligomer Formierung reduzieren und somit die Akkumulation von Amyloidkonglomeraten in zerebralem

Gewebe verhindern beziehungsweise beschränken[74], auch in der Retina finden sich Ablagerungen von Aβ, da dort die ersten Neurone des visuellen Signalweges anatomisch lokalisiert sind. Nicht nur in klassischen neurodegenerativen Erkrankung (z.B. Morbus Alzheimer) spielt dieser Pathomechanismus eine Rolle, sondern auch im Glaukom und der altersabhängigen Makuladegeneration[75]. Weiter verhindert Curcumin die α-Synuklein Aggregation in Morbus Parkinson[74,76].

Zusammenfassend können daher neuroprotektive Effekte von Curcumin auf die menschliche Retina angenommen werden. Weiter wissen wir, dass an verschiedenen neuronalen Zentren in dem visuellen Signalweg ein glaukomatöser Schaden nachweisbar ist[77,78].

Eine kausale Therapie bedarf daher einem systemischen Ansatz, der die Eigenschaften aufweist, auf verschiedene neuronale Zellstrukturen und deren Umgebungsgewebe einwirken zu können. Curcumin bietet eine mögliche therapeutische Strategie, die in vielen in vivo und in vitro Studien nachgewiesen wurde. Weiter verspricht das Potential des Polyphenols Curcumin einen vielseitigen klinischen Einsatz in neurodegenerativen Erkrankungen mit einem chronisch-progredientem Verlauf.

1.2.2. Die Rolle des Wirkstoffes Coenzym Q10

Die mitochondriale Dysfunktion spielt eine grundlegende Rolle in der Apoptose der retinalen Ganglionzelle und Auftreten des axonalen Schadens[5,69]. Durch Herabsetzen der mitochondrialen Effizienz im Alter wird der Funktionsablauf der Organelle anfällig für andere Schadensfaktoren[5,69,79]. Weiter wurde ein gehäuftes Auftreten von genetischen Mutationen, die zu einer mitochondrialen Dysfunktion führen, in Offenwinkelglaukom Patienten beobachtet[80,81]. Mutationen im OPA 1 (optic atrophy 1) Protein führen zu der dominant vererbten Optikusatrophie[81]. OPA 1 ist für die Aufrechterhaltung der Cristae Struktur des Mitochondrions zuständig[82]. In POWG Patienten wurde eine herabgesetzte Expression dieses Proteins gefunden[82,83]. Eine Hochregulierung des OPA 1 Proteins hat einen protektiven Effekt auf das Überleben der retinalen Ganglienzellen in einem Mausmodell[82].

Die Struktur des CoQ10 ist wie folgt aufgebaut: Es handelt sich um ein Chinon-Derivat mit lipophiler Isoprenoid-Seitenkette, mit ähnlichem Aufbau wie Vitamin E[84]. Diese Seitenkette erlaubt eine Anlage an die hydrophoben Plasmamembranen, die die Struktur der Mitochondrien formen. Der menschliche Körper kann CoQ10 selbst produzieren. Es ist ein essentielles Coenzym der oxidativen Phosphorylierung[85,86]. 90% der ATP Synthese wird über diesen Weg

produziert. CoQ10 ermöglicht einen Elektronen - und Protonentransport zwischen Komplex I und II zu dem Komplex III der Atmungskette[85,86]. CoQ10 konnte in der Retina nachgewiesen werden[87].

Die bedeutendste Eigenschaft des Moleküls ist sein anti-oxidativer Charakter[86]. Neben Vitamin C und E, Glutathion und Liponsäure ist CoQ10 einer der wichtigsten Antioxidantien in humanen Zellen. Die Konzentration des Proteins CoQ10 korreliert stark mit optimal arbeitenden, gesunden Mitochondrien[79]. Seine Konzentration nimmt physiologisch altersabhängig im ganzen Körper ab[87].

In der Pathogenese retinaler degenerativer Erkrankungen wie dem Glaukom und der altersabhängigen Makuladegeneration spielt oxidativer Stress eine fundamentale Rolle[79,87]. In einer aktuellen Vergleichsstudie von Ozates et al. konnte gezeigt werden, dass ein Marker für oxidativen Stress (Superoxid Dismutase) in Kammerwasserproben im PEX-Glaukom deutlich erhöht waren im Vergleich zu PEX-Glaukom Patienten, die topisch zweimal täglich 100mg CoQ10 über einen Monat erhalten haben[88]. Es konnten also anti-oxidative Eigenschaften intraokular schon nach einem sehr kurzen Behandlungszeitraum nachgewiesen werden.

In verschiedenen Glaukommodellen (OHT, partielle Sehnervdurchtrennung) zeigt eine systemische CoQ10 Therapie einen protektiven Charakter auf die retinalen Ganglienzellen.

Zusätzlich wurde berichtet, dass eine CoQ10 Therapie zu einer verbesserten Visusleistung in Patienten mit altersabhängiger Makuladegeneration führt[87].

CoQ10 kann insbesondere einen neuroprotektiven Schutz anbieten, der in zahlreichen Forschungsgruppen nachgewiesen wurde[67,87–92]. CoQ10 stellt vor allem eine vielversprechende therapeutische Strategie zur Reduktion der Glutamat-Exzitoxizität und des oxidativen Stresses in der glaukomatösen Neurodegeneration dar[89].

1.3. Wissenschaftliche Fragestellungen

Die folgenden Fragestellungen wurden aufgestellt, um die Ergebnisse und Auswirkungen objektiver Befunde an der Schnittstelle neuroprotektiver und drucksenkender Therapien im Bereich der Glaukomerkrankung leichter einordnen zu können und ihren Stellenwert in der Behandlung von Glaukompatienten zu definieren. Die nachfolgenden Studien wurden in die Diskussion dieser Habilitationsschrift eingeschlossen:

1. Langzeitergebnisse und Komplikationen der Trabectome® Operation in dem adulten, primären Offenwinkelglaukom und Pseudoexfoliationsglaukom

2. Auswirkungen auf die Lebensqualität nach einer invasiven Trabekulektomie und mikro-invasiven Operationstechniken (iStent inject®, Trabectome®)

3. Vergleich der Ergebnisse und Komplikationen zweier minimal-invasiver Verfahren: Goniotomie und Trabectome® Operation im primären Offenwinkelglaukom und Pseudoexfoliationsglaukom

4. Therapeutische Möglichkeiten eines Sekundärglaukoms in Folge einer viralen Uveitis anterior

5. Neuroprotektive Therapieansätze durch die topische Anwendung von Coenzym Q10

6. Neuroprotektive Wirkung von Curcumin in einem Glaukommodell.

2. Originalarbeiten

2.1. Langzeitergebnisse und Komplikationen der Trabectome® Operation in dem adulten, primären Offenwinkelglaukom und Pseudoexfoliationsglaukom

Pahlitzsch M, Davids AM, Zorn M, Torun N, Winterhalter S, Maier AB, Klamann MK, Bertelmann E. Three-year results of ab interno trabeculectomy (Trabectome®): Berlin study group. Graefes Arch Clin Exp Ophthalmol. 2018 Mar;256(3):611-619. https://doi.org/10.1007/s00417-017-3882-8.

Weitere Publikation zu diesem Thema:

- Pahlitzsch M, Gonnermann J, Maier AK, Torun N, Bertelmann E, Joussen AM, Klamann M. [Trabeculectomy Ab Interno in Primary Open Angle Glaucoma and Exfoliative Glaucoma].Klin Monbl Augenheilkd. 2015 Oct;232(10):1198-207.
- Klamann MK, Gonnermann J, Pahlitzsch M, Maier AK, Joussen AM, Torun N, Bertelmann E. iStent inject in phakic open angle glaucoma. Graefes Arch Clin Exp Ophthalmol. 2015 Jun;253(6):941-7. doi: 10.1007/s00417-015-3014-2.

Langzeitergebnisse neu eingeführter Therapieoptionen stellen die wichtigste Grundlage zur der Beurteilung des klinischen Erfolgs dar. Auch zu beachten gilt der Einschluss häufiger Sekundärglaukome, damit die Effektivität und Zuverlässigkeit einer neuen Methode für die verschiedenen Zielgruppen analysiert werden können.

In diese monozentrische Studie wurden 268 POWG und 98 PEX-Glaukom Patienten eingeschlossen.
Der IOD wird durch die Trabectome® Operation von 19,1 mmHg auf 14,3 mmHg im POWG ($p < 0{,}001$) und von 22,5 mmHg auf 14,6 mmHg im PEX-Glaukom ($p < 0{,}001$) in einem Nachbeobachtungszeitraum von drei Jahren gesenkt.
Die topische, antiglaukomatöse Medikation wird von 2,4 auf 1,7 ($p < 0{,}001$) im POWG und von 2,3 auf 1,7 im PEX-Glaukom ($p = 0.006$) reduziert. Entscheidend für den Therapieerfolg ist ein ausführliches präoperatives Gespräch zwischen Arzt und Patient mit Hinweis auf eine weiterbestehende Lokaltherapie. Die Lokaltherapie wirkt sich nachweislich auf die Lebensqualität (QOL) der Glaukompatienten aus, so dass ein Gespräch über die aktuelle QOL in die Entscheidungsfindung präoperativ einbezogen werden sollte.

Der nachfolgende Text entspricht dem Abstrakt des genannten Papers:

Originalsprachiges Abstrakt

„Purpose: To assess the long-term outcome of Trabectome surgery in the treatment of primary open angle glaucoma (POAG) and pseudoexfoliative glaucoma (PEX): 3-year results.

Methods: Trabectome surgery (NeoMedix, Tustin, CA, USA) was performed in 268 POAG patients (women 57.46%, men 42.54%, age 72.35 ± 9.63 years) and 98 PEX glaucoma patients (women 58.16%, men 41.84%, age 73.42 ± 8.54 years), and uncontrolled intraocular pressure (IOP). Parameters were examined preoperatively, 1 day, 6 weeks, 3, 6, 12, 24 and 36 months post surgery. Kaplan-Meier analysis was performed using Criteria A (IOP ≤ 21 mmHg or ≥20% reduction from preoperative IOP), Criteria B (IOP ≤ 18 mmHg or ≥20% IOP reduction), Criteria C (IOP ≤ 21 mmHg, with or without medication) and D (IOP ≤ 18 mmHg, with or without medication). Complete success was defined as IOP ≤ 21 mmHg (Criteria E) and IOP ≤ 18 mmHg without medication (Criteria F).

Results: IOP was reduced from 19.10 ± 4.11 mmHg to 14.27 ± 2.93 mmHg ($p < 0.001$) and glaucoma medication was decreased from 2.40±0.92 to 1.77±1.00 ($p < 0.001$) in POAG after 36 months. In PEX, IOP decreased from 22.49±9.40 mmHg to 14.57±5.05 mmHg after 36 months ($p < 0.001$). Medications dropped from 2.31±1.02 to 1.75±0.91 ($p = 0.006$). Kaplan-Meier analysis showed a success rate of 80.5% for POAG and 80.8% for PEX using criteria A ($p = 0.933$) and 62.4% for POAG and 73.7% for PEX using criteria B ($p = 0.147$) at 36 months

postoperatively. Complete success showed a low survival rate (criteria E- 13.5% in POAG and 7.9% in PEX, p = 0.070 and criteria F- 12.8% in POAG and 5.9% in PEX, p = 0.083).

Conclusions: Trabectome is a safe method to lower IOP in patients with POAG and PEX glaucoma in the long-term period. It is beneficial to inform patients prior to surgery about adjuvant glaucoma medication after the surgery.

2.2. Auswirkungen auf die Lebensqualität nach einer invasiven Trabekulektomie und mikro-invasiven Operationstechniken (iStent inject®, Trabectome®)

Pahlitzsch M, Klamann MK, Pahlitzsch ML, Gonnermann J, Torun N, Bertelmann E. Is there a change in the quality of life comparing the micro-invasive glaucoma surgery (MIGS) and the filtration technique trabeculectomy in glaucoma patients? Graefes Arch Clin Exp Ophthalmol..2017 Feb; 255(2):351-357. https://doi.org/10.1007/s00417-016-3550-4.

Weitere Publikation zu diesem Thema:

- Gonnermann J, Bertelmann E, Pahlitzsch M, Maier-Wenzel AB, Torun N, Klamann MK. Contralateral eye comparison study in MICS & MIGS: Trabectome® vs. iStent inject®. Graefes Arch Clin Exp Ophthalmol. 2017 Feb;255(2):359-365. doi: 10.1007/s00417-016-3514-8.
- Davids AM, Pahlitzsch M, Boeker A, Torun N, Bertelmann E, Maier-Wenzel AK, Hager A, Gonnermann J, Klamann M. iStent inject as a reasonable alternative procedure following failed trabeculectomy? Eur J Ophthalmol. 2018 Mar doi: 10.1177/1120672117747010.

Ein wichtiger Parameter, den es bei der Einführung neuer Therapieverfahren zu evaluieren gilt, ist die QOL eines Patienten. In dieser Studie werden die Auswirkungen auf die QOL durch drei verschiedene invasive und minimal-chirurgische Glaukom-Operationen von 88 POWG Patienten analysiert: 43 Trabectome® Operationen (Alter 72,8 ± 8,8J, weiblich 59,5%, männlich 40,5%), 20 iStent inject® Implantationen (Alter 68,6 ± 16,4J, weiblich 60%, männlich 40%) und 25 TE Prozeduren (Alter 74,2 ± 9,1J., weiblich 58,3%, männlich 41,7%) wurden durchgeführt.

Der National Eye Institute-Visual Function Questionnaire (VFQ-25) wird verwendet, um die Lebensqualität 6 Monate postoperativ zu beurteilen. Die folgenden 12 QOL Parameter werden bewertet: Allgemeine Gesundheit, Augenschmerzen, Sehvermögen, Nah- und Fern-Aktivitäten, psychische Gesundheit, soziale Funktion, Schwierigkeiten des eigenen Rollenverständnisses, Abhängigkeit von anderer Hilfe, Autofahren, Farbsehen und das periphere Sehen.

Es gibt keinen signifikanten Unterschied zwischen der TE und MIGS in Bezug auf die Lebensqualität 6 Monate postoperativ. Die Drucksenkung präsentiert sich signifikant stärker in der TE Kohorte im Vergleich zu MIGS 6 Wochen und 3 Monate postoperativ (p=0,046 und

p=0,046). Die Anzahl der Medikamente wird durch die TE signifikant im Vergleich zu MIGS gesenkt (p<0,001). In dieser Studie kann die QOL durch alle drei Operationstechniken aufrechterhalten werden. Jedoch muss zusammenfassend erwähnt werden, dass die TE zu einer geringeren Einnahme von topischen Medikamenten führt. Dieser Faktor wirkt sich sicher auf die QOL aus, auch wenn er nicht Gegenstand des NEI-VFQ-25 Fragebogens ist. Die Entscheidung, welche chirurgische Technik im Einzelfall gewählt wird, sollte unter Beachtung der einzelnen QOL Kategorien, IOD und Glaukomtherapie getroffen werden.

Der nachfolgende Text entspricht dem Abstrakt des genannten Papers:

Originalsprachiges Abstrakt

„Purpose: This study was conducted to assess the impact on the Quality of Life (QOL) of micro-invasive glaucoma surgery (MIGS: iStent, Trabectome) and a penetrating technique such as Trabeculectomy (TE).

Methods: This study evaluated 88 eyes of 88 open angle glaucoma patients undergoing glaucoma surgery: 43 (mean age 72.8 ± 8.8y, female 59.5 %, male 40.5 %) Trabectome (NeoMedix, Inc., Tustin, CA, USA), 20 (mean age 68.6 ± 16.4y, female 60 %, male 40 %) iStent (Glaucos Corporation, Laguna Hills, CA, USA), and 25 TE patients (mean age 74.2 ± 9.1y female 58.3 %, male 41.7 %). The National Eye Institute-Visual Functioning Questionnaire (VFQ-25) survey was used to assess the QOL at 6 months post surgery. The following 12 QOL parameters were evaluated: general health, ocular pain, general vision, near and distance activities, mental health, social functioning, role difficulties, dependency, driving, color vision, and peripheral vision. Intraocular pressure (IOP), number of topical medications, and visual acuity (VA) were examined preoperatively, 1 day, 6 weeks, 3 months, and 6 months post surgery. Statistical data were calculated using SPSS (v20.0, SPSS, Inc.).

Results: There was no significant difference between TE and MIGS in the quality of life 6 months postoperatively. IOP was significantly lower in TE compared to MIGS at 6 weeks and 3 months postoperatively (p = 0.046 and p = 0.046). Number of medications was significantly decreased in TE compared to MIGS (p < 0.001). A significant difference in VA between TE and MIGS could be assessed at day 1 post-op (p = 0.011).

Conclusion: In this study cohort, the QOL can be maintained by all three surgical techniques. Patients, however, need lower numbers of topical medication in TE, which would impact QOL

even though it is not included in the NEI-VFQ-25. The decision of the most appropriate surgical technique should be made by including single QOL categories, IOP and glaucoma medication outcome."

2.3. Vergleich der Ergebnisse und Komplikationen zweier minimal-invasiver Verfahren: Goniotomie und Trabectome® Operation im primären Offenwinkelglaukom und Pseudoexfoliationsglaukom

Pahlitzsch M, Gonnermann J, Maier AK B, Bertelmann E, Klamann MKJ, Erb C. Modified goniotomy as an alternative to trabectome in primary open angle glaucoma and pseudoexfoliation glaucoma: 1 year results. Canadian Journal of Ophthalmology / Journal Canadien d'Ophtalmologie. 2017 Feb;52(1):92-98. https://doi.org/10.1016/j.jcjo.2016.07.011.

Ein weiteres Therapieverfahren mit einem neuwertigen, modifizierten Ansatz einer vorhandenen Technik wird in dieser Arbeit mit einer modernen MÍGS Operation verglichen. Der Vergleich zweier aktueller MIGS Prozeduren schließt dabei nicht nur das POWG, sondern auch das häufigste Sekundärglaukom, das PEX-Glaukom, ein. Es handelt sich um die modifizierte Goniotomie und Trabekulotomie ab interno (Trabectome®). Die Goniotomie ab interno bietet ein kostengünstiges Verfahren, das sich in ihrer Urform seit Jahrzehnten als glaukomchirurgisches Verfahren vor allem für das kongenitale Glaukom etabliert hat. Der operative Effekt ist dem Trabectome® Eingriff ähnlich, in dem mit einem speziellen Goniotomiemesser das Trabekelmaschenwerk eröffnet wird. Der Nachteil der klassischen Goniotomie ab interno liegt in der unberechenbaren Augendrucksenkung, da die Goniotomie in der Regel über 180° erfolgt und zum Teil mit einer größeren Zyklodialyse verbunden ist. Aufbauend aus diesen Erfahrungen ist eine modifizierte Variante der Goniotomie ab interno entwickelt worden. Hierbei wird eine Goniotomie über 5 Uhrzeiten durchgeführt und mit einer nur über 1 Stunde reichenden Zyklodialyse kombiniert, analog der Erfahrung mit suprachoroidalen Stents.

68 POWG Patienten (Alter 65,7 Jahre) und 22 PEX-Glaukom Patienten (Alter 78,3 Jahre) wurden mittels Goniotomie operiert. Die Trabectome® Vergleichsgruppe setzt sich aus 119 POWG (73,9 Jahre) und 27 PEX-Glaukom Patienten (75,2 Jahre) zusammen.

In der modifizierten Goniotomie POWG Gruppe zeigt sich eine IOD Reduktion um 5,8 mmHG ($p < 0{,}001$) und im PEX-Glaukom um 6,7 mmHg ($p=0{,}004$) 1 Jahr postoperativ. In der Trabectome® POWG Gruppe fällt der IOD dementsprechend um 4,6 mmHg ($p<0{,}001$), in der PEX Kohorte um 9,7 mmHg ($p=0{,}002$). Die Glaukomtherapie sinkt im POWG um 1,1 Medikamente ($p<0{,}001$), im PEX-Glaukom um 0,7 auf 1,9 Medikamente ($p=0{,}035$) in der Goniotomie Kohorte. In der Trabectome® Gruppe werden die Therapeutika um 0,25 ($p=0{,}034$) im POWG und um 0,56 im PEX-Glaukom ($p=0{,}033$) 1 Jahr postoperativ reduziert.

Zusammenfassend kann eine signifikante Drucksenkung und Reduktion der Anzahl der antiglaukomatösen Medikamente durch die alternative Technik „modifizierte Goniotomie“ in einer adulten POWG- und PEX- Glaukomkohorte nach einem Jahr Nachbeobachtung erreicht werden. Beide Verfahren stellen eine effektive und komplikationsarme Möglichkeit in der Behandlung milder bis moderater primärer und sekundärer Offenwinkelglaukome dar - ohne signifikanten Unterschied zugunsten eines chirurgischen Verfahrens.
Interessant sind vor allem auch die Ergebnisse moderner MIGS Methoden bezüglich der Sekundärglaukome und vorhandener Behandlungsnischen, so dass nach Feststellung einer effizienten Therapie im POWG je nach Operationstechnik andere Glaukomtypen betrachtet werden sollten.

Der nachfolgende Text entspricht dem Abstrakt des genannten Papers:

Originalsprachiges Abstrakt

„Objective: To assess the outcome of modified goniotomy and trabeculotomy ab interno (Trabectome) surgery in adult primary open-angle glaucoma (POAG) and pseudoexfoliation (PEX) glaucoma.

Design: Retrospective cohort outcome study.

Participants: Two hundred and thirty-six eyes of 236 patients.

Methods: This cohort outcome study included 68 POAG (mean age: 65.7 ± 16.0 years) and 22 PEX glaucoma patients (mean age: 78.3 ± 7.9 years) in the modified goniotomy cohort and 119 POAG (mean age: 73.9 ± 9.6 years) and 27 PEX glaucoma patients (mean age: 75.2 ± 8.0 years) in the Trabectome cohort. Modified goniotomy is defined as combined ab interno cyclodialysis and goniotomy. The patients were followed up for 12 months, and we analysed the data using SPSS v19.0.

Results: In POAG, the intraocular pressure (IOP) was significantly reduced by 4.6 mm Hg in the Trabectome cohort ($p < 0.001$) and by 5.8 mm Hg ($p < 0.001$) in the goniotomy group at 1-year follow-up. In PEX glaucoma, the mean IOP was reduced by 9.7 mm Hg ($p = 0.002$) in the Trabectome surgery and by 6.7 mm Hg ($p = 0.004$) in the goniotomy cohort 1 year later. Comparing both surgery techniques in POAG, no significant correlation was found in terms of IOP at any of the follow-up visits (IOP at 1 year, $p = 0.553$). In PEX glaucoma, the IOP, visual

acuity, and number of glaucoma medications did not differ significantly between the 2 surgery techniques 1 year later (IOP: $p = 0.300$; VA: $p = 0.391$; therapy: $p = 0.908$).

Conclusion: Modified goniotomy and Trabectome surgery are reliable and effective tools for the management of moderate POAG and PEX glaucoma. There was no significant difference in IOP between the 2 procedures over a follow-up period of 1 year.”

2.4. Therapeutische Möglichkeiten eines Sekundärglaukoms in Folge einer viralen Uveitis anterior

Pohlmann D , Pahlitzsch M (=shared first) , Stephan Schlickeiser , Metzner S , Lenglinger M, Bertelmann E , Maier AK B , Winterhalter S, Pleyer U. Virus-associated anterior uveitis and secondary glaucoma: Diagnostics, clinical characteristics, and surgical options. PLoS One. 2020 Feb 24;15(2):e0229260. doi: 10.1371/journal.pone.0229260.

Weitere Publikation zu diesem Thema:

- Pahlitzsch M, Torun N, Gonnermann J, Maier AK, Pleyer U, Bertelmann E, Joussen A, Klamann MK. Trabeculectomy ab interno (trabectome): Yet another possibility in the treatment of uncontrolled glaucomatocyclitic crisis under systemic valganciclovir therapy? Eye (Lond). 2015 Oct;29(10):1335-9.

Neben dem PEX-Glaukom und Pigmentdispersionsglaukom spielt das entzündliche Sekundärglaukom eine wichtige Rolle in dem klinischen Alltag und stellt spezielle Anforderungen an den betreuenden Arzt. Neben einer Stabilisierung der Entzündungssituation ist eine umfassende medikamentöse und chirurgische drucksenkende Therapie notwendig, so dass immer neue Therapieansätze evaluiert werden.
Eine virale anteriore Uveitis macht 4-9% aller Uveitiden in einem Tertiärzentrum aus. In diese Studie wurden 270 Patienten (n=57 Cytomegalievirus, n=77 Herpes simplex Virus, n=45 Varizella zoster Virus, n=77 Rubella Virus, n=14 Multiple Viren) eingeschlossen und die Auswirkungen der inflammatorischen Grunderkrankung auf die Entstehung und Therapie eines Sekundärglaukoms untersucht.
52 Patienten (19%) entwickeln nach dem Erkrankungsbeginn ein Sekundärglaukom. 27 (10%) benötigen eine Glaukomoperation zur Einstellung ihres individuellen Zieldrucks bei nicht ausreichender Drucksenkung durch topische und orale Medikation. Weiter müssen sich 10 Patienten einem Zweiteingriff bei Therapieversagen der ersten Operation unterziehen (n=9 MIGS, n=1 TE).
Zusammenfassend stellt die Trabekulektomie zur Behandlung eines uveitischen Sekundärglaukoms weiterhin den Goldstandard dar. MIGS zeigt kurzfristig eine zuverlässige Druckreduktion. Diese kann in Notfallsituationen wie einem akuten Schub einer Uveitis gut

genutzt werden, um schwerwiegende Komplikationen einer filtrierenden Operation zu verhindern.

Der nachfolgende Text entspricht dem Abstrakt des genannten Papers:

Originalsprachiges Abstrakt

„In this retrospective, single-center, observational study, we compared the clinical characteristics, analyzed the glaucoma development, and the glaucoma surgery requirement mediators in patients with different virus-associated anterior uveitis (VAU). In total, 270 patients (= eyes) with VAU confirmed by positive Goldmann-Witmer coefficients (GWC) for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus (VZV), rubella virus (RV), and multiple virus (MV) were included. Clinical records of these patients were analyzed. Demographic constitution, clinical findings, glaucoma development, and surgeries were recorded. The concentrations of 27 immune mediators were measured in 150 samples of aqueous humor. The GWC analysis demonstrated positive results for CMV in 57 (21%), HSV in 77 (29%), VZV in 45 (17%), RV in 77 (29%), and MV in 14 (5%) patients. CMV and RV AU occurred predominantly in younger and male patients, while VZV and HSV AU appeared mainly with the elderly and females ($P<0.0001$). The clinical features of all viruses revealed many similarities. In total, 52 patients (19%) showed glaucomatous damage and of these, 27 patients (10%) needed a glaucoma surgery. Minimal-invasive glaucoma surgery (MIGS) showed a reliable IOP reduction in the short-term period. In 10 patients (37%), the first surgical intervention failed and a follow-up surgery was required. We conclude that different virus entities in anterior uveitis present specific risks for the development of glaucoma as well as necessary surgery. MIGS can be suggested as first-line-treatment in individual cases, however, the device needs to be carefully chosen by experienced specialists based on the individual needs of the patient. Filtrating glaucoma surgery can be recommended in VAU as an effective therapy to reduce the IOP over a longer period of time.“

PLOS ONE

RESEARCH ARTICLE

Virus-associated anterior uveitis and secondary glaucoma: Diagnostics, clinical characteristics, and surgical options

Dominika Pohlmann[1‡*], Milena Pahlitzsch[1‡*], Stephan Schlickeiser[2], Sylvia Metzner[1], Matthias Lenglinger[1], Eckart Bertelmann[1], Anna-Karina B. Maier[1], Sibylle Winterhalter[1], Uwe Pleyer[1]

1 Berlin Institute of Health, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin, Germany, **2** Institute of Medical Immunology, Charité–University Medicine, Berlin, Berlin, Germany

‡ These authors share first authorship on this work.
* dominika.pohlmann@charite.de (DP); milena.pahlitzsch@charite.de (MP)

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Abstract

In this retrospective, single-center, observational study, we compared the clinical characteristics, analyzed the glaucoma development, and the glaucoma surgery requirement mediators in patients with different virus-associated anterior uveitis (VAU). In total, 270 patients (= eyes) with VAU confirmed by positive Goldmann-Witmer coefficients (GWC) for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus (VZV), rubella virus (RV), and multiple virus (MV) were included. Clinical records of these patients were analyzed. Demographic constitution, clinical findings, glaucoma development, and surgeries were recorded. The concentrations of 27 immune mediators were measured in 150 samples of aqueous humor. The GWC analysis demonstrated positive results for CMV in 57 (21%), HSV in 77 (29%), VZV in 45 (17%), RV in 77 (29%), and MV in 14 (5%) patients. CMV and RV AU occurred predominantly in younger and male patients, while VZV and HSV AU appeared mainly with the elderly and females (P<0.0001). The clinical features of all viruses revealed many similarities. In total, 52 patients (19%) showed glaucomatous damage and of these, 27 patients (10%) needed a glaucoma surgery. Minimal-invasive glaucoma surgery (MIGS) showed a reliable IOP reduction in the short-term period. In 10 patients (37%), the first surgical intervention failed and a follow-up surgery was required. We conclude that different virus entities in anterior uveitis present specific risks for the development of glaucoma as well as necessary surgery. MIGS can be suggested as first-line-treatment in individual cases, however, the device needs to be carefully chosen by experienced specialists based on the individual needs of the patient. Filtrating glaucoma surgery can be recommended in VAU as an effective therapy to reduce the IOP over a longer period of time.

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Introduction

Virus-associated anterior uveitis (VAU) is caused by cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus (VZV), and rubella virus (RV). The most common VAU is the herpetic cause which includes CMV, HSV, and VZV and accounts for 5% to 10% of all uveitis cases seen at tertiary referral centers.[1–4] In most cases, the diagnosis is made based on clinical characteristics. Even though the individual viruses may show some subtle differences, there are several overlapping signs which make the diagnosis challenging. Although the aqueous humor (AH) can be easily examined by polymerase chain reaction (PCR) or Goldmann-Witmer coefficient (GWC), the confirmation of the virus in AH is often not done. Additionally, prior studies reported that reverse transcription (RT)—PCR has frequently failed to demonstrate the presence of RV RNA in Fuchs'Uveitis Syndrome (FUS) due to a low-viral load below detection level and a high-rate of anti-RV antibodies which block the viral replication.[5–9] For the analysis of immunoglobulin fraction in the AH and serum, the use of GWC is mandatory to differentiate RV, but it is also mandatory for all other herpetic viruses especially in a period of latency. Previous results showed that immune mediators play a crucial role in specific viral inflammation and influence intraocular pressure (IOP). CMV demonstrated a stronger active inflammatory response, while RV may trigger chronic inflammation.[10] Inflammatory effects on the IOP levels differ between the virus types. CMV is a well-known cause of secondary glaucoma with high IOP levels >30mmHg,[11] first described by Posner and Schlossman in 1948.[12–14] Approximately 10–40% of VAU patients could develop glaucoma.[15–20] One very important risk factor for the development of chronic glaucoma is the number of IOP peaks.[15] Additionally, patients developing glaucoma usually present themselves with high IOP levels at their first inflammatory episode.[15] The amount of the viral load is also significantly associated with the number of uveitic recurrences.[21] Finally, 19% of VAU patients (VZV and HSV) needed surgical intervention to control individually elevated IOP levels.[15]

In this study, we examined 270 patients (= eyes) with VAU of which 52 developed secondary glaucoma. The diagnosis was made based on the detection of CMV, HSV, VZV, and RV to compare their demography and clinical characteristics. We place an emphasis on the IOP development and glaucoma therapy, considering glaucoma medication and different surgical therapeutic approaches. In addition, we measured immune mediators in AH of 150 eyes to add more rigor to the clinical findings.

Methods

The retrospective, single-center study design complies with the ethical principles for medical research as outlined in the Declaration of Helsinki approved by the local ethics committee (EA 4/054/16) of Charité University Medicine Berlin. From January 2009 to December 2018, a total of 270 immunocompetent patients (= eyes) with CMV (57), HSV (77), VZV (45), RV (77), and multiple virus (MV) (14) were included. For routine diagnostic purpose, AH samples were obtained from all patients to analyze the antibody synthesis by GWC as described previously.[10] Patients with more than one positive virus of the aforementioned viruses were summarized in the group MV. The patients gave informed consent before anterior chamber (AC) stab incision and glaucoma surgery. Data were fully anonymized before analysis.

The following clinical characteristics were collected from patient´s medical records before the AC stab incision and in patients who underwent glaucoma surgery pre- and postoperatively: unilateral or bilateral uveitis, acute or chronic course, previous keratitis, visual acuity (VA) in log of the Minimum Angle (logMAR), IOP, conjunctival redness, corneal edema, keratic precipitates (KP), character of KPs, location of KPs, cells in AC, hypopyon, fibrin, iris

synechia, irisatrophy, heterochromia, lens status, vitreous involvement, and macular edema. Inflammation was evaluated using scoring criteria set out by the Standardization of Uveitis Nomenclature (SUN) working group.[22] Macular edema was detected by optical coherence tomography (SPECTRALIS® Heidelberg Engineering, Heidelberg, Germany). Additionally, glaucoma therapy, as well as the systemic antiviral therapy were documented. Apart from the topical steroids, patients with HSV received oral acyclovir (400mg / 5x day for 4–6 weeks in acute course; 400mg / 3x day as maintenance dose) and VZV patients were treated with a higher dose of acyclovir (800mg / 5x day for 4–6 weeks; 400mg / 3x day as maintenance dose). In cases of side-effects or non-response, the therapy was switched to oral valacyclovir (HSV: 500 to 1000mg twice a day; VZV: 1000mg 3x day). Patients with CMV received 900 mg valgancyclovir twice a day for two weeks; 450mg twice a day for 3–6 months. Elevated IOP >21mmHg was treated with topical anti-glaucomatous therapy and IOP >30mmHg additionally with oral acetazolamide.

Goldmann-Witmer coefficient

The AH samples were immediately processed after the AC stab incision. A modified ELISA technique (Enzynost®, Dade Behring Marburg, Germany) was performed to detect antibodies in AH and serum, diluted to an IgG level of 1 mg/dL after total IgG in the serum and AH were measured.[6,23] A comparison of photometric signals of $\Delta E > 0.2$ allowed for detection of intraocular IgG antibodies to CMV, HSV, VZV, and RV. The antibody index (AI) was determined using the GWC.[23,24] The diagnosis was confirmed by $AI > 3.0$ or $\Delta E > 0.200$ for all viruses.

Immune mediator analysis

From 270 AH samples, we were able to measure the concentration of immune mediators in 150 AH samples by Bio-Plex ProTM magnetic color-bead-based multiplex assay (Bio-Rad Laboratories, Inc. Hercules, CA). Fifty microliters of AH were used for the measurement. Samples with insufficient material could not be measured. The following 27 immune mediators were analyzed: eotaxin, fibroblast growth factor basic (FGFbasic), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin-1 receptor antagonist (IL-1RA), IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, interferon-gamma (IFN-γ), interferon gamma-induced protein 10 (IP-10), macrophage inflammatory proteins 1 alpha and beta (MIP-1α and MIP-1β), monocyte chemotactic protein 1 (MCP1), platelet-derived growth factor (PDGF), regulated upon activation normal T cell expressed and secreted (RANTES), tumor-necrosis-factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF). The assay was conducted according to manufacturer's instruction. Data analysis was performed by Bio-Plex Manager TM software 1.1.

Glaucoma criteria

Glaucoma was defined by an optic neuropathy showing characteristic glaucomatous optic disc alterations and visual field defects. A major risk factor was IOP elevation. All patients included in this study demonstrated an open chamber angle in the gonioscopy (Shaffer III-IV, well-pigmented trabecular meshwork, no neovascularization). The optic disc was classified using the diagnostic criteria described by Jonas.[25] The inclusion criteria entailed best-corrected visual acuity of at least 20/200 and informed patient consent for surgery, if necessary. The intraocular pressure (IOP) was measured by using the well-known Goldmann applanation tonometry.[26] Preoperatively and in the subsequent visits post-surgery IOP readings, VA in logMAR and the number of glaucoma medications were analyzed. Success rate of therapy is defined by a

controlled IOP and inflammatory situation which might differ in individuals due to their severity of glaucomatous damage. Failure rate was defined by a second intervention due to uncontrolled individual pressure in secondary glaucoma. Patients attended clinics preoperatively as well as follow-ups one day, six weeks, three months, six months, one year, and two years postoperatively.

Glaucoma surgery

First line surgical therapy was minimal-invasive glaucoma surgery (MIGS) including Trabectome® surgery and iStent inject® implantation. Surgical interventions were always conducted under stable inflammatory conditions. Furthermore, we considered the canaloplasty as a first line procedure (MIGS) to reduce IOP. Two patients received cyclophotocoagulation (inferior hemisphere, 20 spots, 2000 mW, 2000 mseconds) in our retrospective data set. Second line therapy involved filtrating glaucoma surgery such as trabeculectomy. The surgeries were conducted by two surgeons (SW, EB).

Minimal-invasive glaucoma surgery

Surgical scheme of MIGS in short[27–30]: a 1.8 mm incision in the limbal temporal cornea was made, acetylcholine chloride 1% (Miochol) was inserted individually as needed and followed by an injection of ophthalmic viscosurgical solution to contain the AC morphology. At this point, there are two options. First option–the Trabectome® (NeoMedix, Inc., Tustin, CA, USA) handpiece was inserted and the selective electrosurgical ablation was activated to remove an 120˚ arc of trabecular meshwork and an inner wall of the Schlemm canal.[27,30] Alternatively, iStent inject® (Glaucos Corporation, Laguna Hills, CA, USA) implantation was chosen and two iStents were implanted through the nasal trabecular meshwork into Schlemm's canal, usually separated by two hours.[28,29] MIGS were conducted under gonioscopic view.

Canaloplasty ab externo

Lewis et al. published this surgical procedure in detail.[31] In brief, after conjunctival limbal opening at the upper quadrant, a non-penetrating two-flap dissection of the sclera was prepared to expose Schlemm's canal.[31] The iTrack-microcatheter (Ellex iScience Inc., Fremont, CA, USA) was used to dilate the full circumference of the canal with the assistance of sodium hyaluronate (Healon GV, Advanced Medical Optics, Inc., Santa Ana, CA, USA). Catheterization was conducted over the complete circumference. A 10-0 Prolene suture (Ethicon, Inc., Somerville, NJ, USA) was then applied to the microcatheter tip and left in the canal with both ends tightened to expand the trabecular meshwork inward.[31,32]

Filtrating surgery

Trabeculectomy can be used as first or second line therapy to reduce high-levels of uncontrolled IOP with use of Mitomycin C (0.2 mg/ml). In essence, a fornix-based peritomy of conjunctiva in the upper quadrant was created and an approximately 2.5 × 2.5 mm scleral flap was dissected, [33–35] followed by a Mitomycin C (0.2 mg/ml) sponge application on the scleral surface for three minutes before lavage with generous balanced salt solution (BSS). Intralamellar scleral sutures using 10–0 nylon (Alcon, Camberley, UK) were pre-placed at the corners of the scleral flap, and a paracentesis was placed temporarily. Furthermore, a sclerostomy (500μm) was created with a Khaw Descemet membrane punch 7–101 (Duckworth & Kent, Baldock, UK) and a surgical iridectomy was conducted. Finally, the pre-placed sutures were tightened, and the conjunctival tissue closed.[33–35]

Statistical analysis

Data of clinical characteristics and cytokine concentrations were analyzed by using GraphPad Prism 8 (GraphPad Software, La Jolla, CA) and SPSS (Version 20.0). For demographic and clinical parameters descriptive statistics (mean, standard deviation), Chi-Square and Fisher test were performed. For differences in cytokine concentrations, non-parametric Mann-Whitney testing was performed. Two-tailed, non-parametric Spearman method was applied to assess the correlation between variables. Comparison of preoperative to postoperative glaucoma parameters was conducted by the independent sample t-test. Numeric variables, which do not show a normal distribution range, were compared with the Mann–Whitney U test (two cohorts) and the Kruskal-Wallis test (three cohorts). Spearman´s correlation was analyzed to correlate AI coefficient of all virus types and IOP. For testing normality, the Kolmogorov-Smirnov-Test was applied. A p-value of <0.05 indicated a statistically significant difference.

Results

Demography

The GWC analysis demonstrated positive results for CMV in 57 (21%), for HSV in 77 (29%), for VZV in 45 (17%), for RV in 77 (29%), and MV (8 VZV+HSV; 2 VZV+CMV; 2 HSV +CMV; 1 VZV+RV; 1 CMV+RV) in 14 (5%) out of 270 patients (= eyes) (Fig 1). The CMV (median age, 42; range 19–89) and RV (median age, 44 years; range 19–76) patients were younger than the HSV (median age, 56; range 19–87), VZV (median age, 67; range 19–96), and especially the MV cohort (median age, 75; range 28–81) ($P <0.0001$). The male and female ratio did not differ between CMV, HSV, VZV, RV, and MV patients ($P = 0.1029$). Unilateral involvement was typical for almost all viruses (76–98%), but one quarter of VZV (24%) patients also had a bilateral manifestation ($P = 0.0149$). To note, the second eye presented only a keratitis, not a keratouveitis, The course of disease was predominantly acute for CMV (55/57; 96%) patients, while chronic disease was observed in RV (52/77; 84%) and in HSV patients (52/77; 68%) ($P<0.0001$). VZV and MV patients equally demonstrated an acute and a chronic course of disease. Almost half of the patients (150/270; 56%) revealed a previous keratitis, in particular HSV (44/77; 57%) and VZV (23/45; 51%) patients ($P<0.0001$). No VZV patient had a history of previous herpes zoster ophthalmicus (no dermal lesions). The worst VA revealed VZV patients with 0.5 logMAR, followed by MV with 0.3 logMAR, and HSV/RV patients with 0.2 logMAR ($P>0.0001$). The CMV patients had the best VA with 0 logMAR, although they presented the highest median IOP with 27 mmHg compared to VZV (18 mmHg), HSV, RV (16 mmHg), and MV (16.5 mmHg) ($P<0.0001$). The CMV patients also possessed the highest number of local and systemic anti-glaucomatous drugs compared to other patients ($P<0.0001$). More details of demographic data are presented in Table 1.

Clinical findings

The ophthalmologic findings from the preoperative day are found in Table 2. Conjunctival redness was presented more often in CMV (29/57; 54%) and VZV (21/45; 47%) in comparison to RV (21/77; 27%) ($P = 0.0086$; $P = 0.0435$). Corneal edema was observed only in a few patients (1–7%). The occurrence of KPs differed between the groups ($P = 0.04$). HSV (54/77; 70%) and VZV (30/45; 67%) patients showed significant differences compared to RV (67/77; 87%) patients. The character and location of KPs were not reported consistently and therefore could not be analyzed accurately. Iris synechia were especially noted in HSV (12/77; 12%) patients compared to CMV (0/57; 0%) patients ($P = 0.0352$), while iris atrophy was not noted in a specific patient group ($P = 0.3293$). Anterior inflammation was observed in all patients

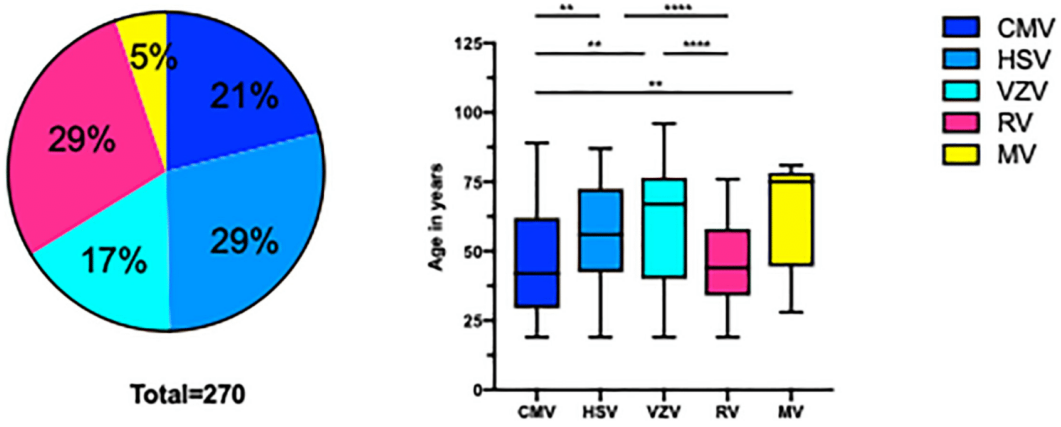

Fig 1. Virus distribution of the complete study cohort and age association of virus infection.

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(P = 0.5991), but VZV patients showed more severe inflammation with hypopyon (2/45; 4%), vitreous haze (8/45; 31%), and cystoid macula edema (CME) (5/45; 11%). In contrast, iris synechia were not present, but iris heterochromia (46/77; 60%) (P<0.0001), cataract at presentation (55/77; 71%), and vitreous haze occurred more frequently (47/77;61%) (P<0.0001) in RV patients.

Glaucoma

In total, 52 patients (= eyes) out of 270 (19%) (mean age 48.0±18.8 years) with VAU, showed glaucomatous damage in accordance to Jonas criteria[15] and were diagnosed with secondary glaucoma (see Table 3 for descriptive statistics). Of these, a total of 27 patients (= eyes) needed glaucoma surgery (10% of all VAU) (RV: 11; CMV: 6; HSV: 5, VZV: 3; MV: 2). In detail, 16 patients (62.8±12.4years) received MIGS including iStent inject® and Trabectome®, in 2 patients (52.5±31.8years) cyclophotocoagulation was performed, and 9 patients (52.1±19.7 years) obtained trabeculectomy and Mitomycin C. Nine VAU patients had a trabeculectomy as first line procedure due to unavailability and missing experience of newly developed MIGS devices in uveitis at that point in time (retrospective data from 01/2009 onwards). Results are outlined in Table 4. IOP preoperatively was reduced from 28.18±9.32mmHg to 14.72 ±6.67mmHg two years postoperatively (P = 0.004). In addition, a significant reduction of glaucoma medication can be reported from preoperatively 1.91±0.94 to 0.81±1.16 two years after surgery (P = 0.040). The trendline chart of IOP and glaucoma medication can be found in Fig 2. Follow-up interventions in individually uncontrolled IOP were carried out in 10 eyes (37%) (IOP >16 mmHg) (Fig 3). The first surgical intervention failed in nine patients receiving MIGS (Trabectome®: 4; iStent inject®: 3; Canaloplasties ab externo: 2) and failed in one patient receiving trabeculectomy and Mitomycin C. No patient with MV needed further surgeries. MIGS did not show any perioperative complications other than blood reflux. Blood reflux can be used as a parameter to assess successful trabecular meshwork surgery. Furthermore, there were no incidences of sustained hypotony, choroidal effusion, hemorrhage, infection, aqueous misdirection, or wound leakage in all surgeries that were carried out. Duration until performed follow-up interventions is outlined in Fig 3.

Table 1. Demographic parameters of all virus-associated anterior uveitis cohorts.

							P Value									
	Total	**CMV**	**HSV**	**VZV**	**RV**	**MV**		**CMV vs HSV**	**CMV vs VZV**	**CMV vs RV**	**CMV vs MV**	**HSV vs VZV**	**HSV vs RV**	**HSV vs MV**	**VZV vs RV**	**VZV vs MV**	**RV vs. MV**
Number of patients (%)	270	57 (21)	77 (29)	45 (17)	77 (29)	14 (5)											
Median age at presentation (range)	52 (19–96)	42 (19–89)	56 (19–87)	67 (19–96)	44 (19–76)	75 (28–81)	**<0.0001**	**0.0022**	**0.0018**	0.7373	**0.0021**	0.3242	**0.0004**	0.1183	**0.0004**	0.3465	**0.0004**
Gender																	
Female (%)	120 (44)	21 (37)	40 (52)	25 (56)	30 (39)	4 (29)	0.1029	0.0826	0.0593	0.8028	0.5616	0.3853	0.1056	0.1074	0.0755	0.0778	0.4598
Male (%)	150 (56)	36 (63)	37 (48)	20 (44)	47 (61)	10 (71)											
Median of Antibody-Index (GWC) (range)	5.6 (1.31–485.5)	7.265 (1.6–485.5)	5.495 (2.1–109.7)	4.330 (1.31–106.6)	6.450 (1.89–199.7)	3.94 (1.91–83.33)	**0.0038**	**0.0423**	**0.0047**	0.6101	**0.0021**	0.1842	0.0972	0.0708	**0.0059**	0.6107	**0.0028**
Unilateral	230 (85)	56 (98)	66 (86)	34 (76)	62 (81)	12 (86)	**0.0149**	**0.012**	**0.0004**	**0.0018**	**0.0368**	0.1591	0.3895	>0.999	0.5183	0.4232	0.6464
Bilateral	40 (15)	1 (2)	11 (14)	11 (24)	15 (19)	2 (14)											
Acute course	123 (46)	55 (96)	25 (32)	23 (55)	10 (16)	7 (50)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.042**	**0.0274**	0.2063	**<0.0001**	0.9421	**0.006**
Chronic course	147 (54)	2 (4)	52 (68)	22 (49)	52 (84)	7 (50)											
Previous keratitis	150 (56)	0 (0)	44 (57)	23 (51)	25 (40)	6 (43)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.5183	**0.0487**	0.320	0.268	0.5895	0.816
Visual acuity median in logMAR (range)	0.2 (0–2.5)	0 (0–2)	0.2 (-0.1–2.5)	0.5 (0–2.5)	0.2 (0–2.5)	0.3 (0–1)	**<0.0001**	**0.0028**	**<0.0001**	**0.0020**	**0.0035**	**0.0028**	0.8261	0.5886	0.0003	0.0520	0.3528
Intra ocular pressure (IOP) median in mmHG (range)	18 (6–58)	27 (10–55)	16 (6–38)	18 (7–40)	16 (8–58)	16.5 (8–42)	**<0.0001**	**<0.0001**	**0.0009**	**<0.0001**	**0.0163**	0.1116	0.8376	0.7248	0.1571	0.7274	0.7913
IOP																	
>21–29 mmHg	81 (30)	27 (47)	15 (19)	14 (31)	22 (29)	3 (21)	**<0.0001**	**0.0002**	0.2419	**0.0007**	0.4906	**0.0099**	0.7416	**0.0037**	**0.0339**	0.0632	**0.0104**
Acute course	48 (59)	27 (100)	8 (53)	6 (43)	5 (23)	2 (67)											
Chronic course	33 (41)	0 (0)	7 (47)	8 (57)	17 (77)	1 (33)											
IOP																	
>30 mmHg	39 (14)	19 (33)	3 (4)	8 (18)	6 (8)	3 (21)	0.3718	0.1487	0.3486	0.4373	0.9286	0.1394	0.5	0.2	0.7299	0.2606	0.2976
Acute course	25 (64)	19 (100)	1 (33)	3 (38)	0 (0)	2 (67)											
Chronic course	14 (36)	0 (0)	2 (67)	5 (62)	6 (100)	1 (33)											
Number of local antiglaucomatous eye drops							**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.1487	0.2645	0.3967	0.6740	0.2628	**0.042**	0.3138
0	125	10	38	27	45	5											
1	50	5	16	10	16	3											
2	61	21	18	8	10	4											
3	34	21	5	0	6	2											
Systemic acetazolamide																	
yes	222 (82)	34 (60)	69 (90)	35 (78)	71 (82)	10 (71)	**<0.0001**	**<0.0001**	0.052	**<0.0001**	0.4159	0.0754	0.5606	0.0644	**0.0227**	0.6258	**0.0222**
no	48 (18)	23 (40)	8 (10)	10 (22)	6 (8)	4 (29)											

CMV = cytomegalovirus, GWC = Goldmann–Witmer coefficient, HSV = herpes simplex virus, IOP = Intraocular pressure, logMAR = log of the Minimum Angle, MV = multiple virus, RV = rubella virus, VZV = varicella-zoster virus

Kruskal-Wallis test, Chi-square-Test, Mann-Whitney Test were performed.

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Table 5 presents results of the AI coefficient in comparison to glaucoma parameters. We found higher AI values in RV and CMV patients in the IOP >30 mmHg cohort compared to a moderate IOP increase (RV: IOP >30 mmHg, AI 104.8±89.3 vs. IOP <30 mmHg 21.3±42.9). The results are not statistically significant but might be an interesting trend to follow. In addition, no significance was found between AI coefficient and topical/systemic glaucoma therapy (P>0.05). The parameter 'glaucoma surgery´ did not show any statistical significance to AI coefficient in all study cohorts.

Immune mediators

In a total of 150 patients (CMV:23; HSV: 34; VZV:16; RV: 77), 27 immune mediators were measured. The median concentration of all cytokines was similar in all groups, except of six

Table 2. Clinical findings of all virus-associated anterior uveitis cohorts.

						P Value									
	CMV N = 57	**HSV N = 77**	**VZV N = 45**	**RV N = 77**	**MV N = 14**		**CMV vs HSV**	**CMV vs VZV**	**CMV vs RV**	**CMV vs MV**	**HSV vs VZV**	**HSV vs RV**	**HSV vs MV**	**VZV vs RV**	**VZV vs MV**	**RV vs. MV**
Conjunctival redness																
	29	28	21	21	6	0.0813	0.0929	0.4224	**0.0086**	0.5907	0.2627	0.3018	0.6441	**0.0435**	0.8027	0.2867
no	(54)	(36)	(47)	(27)	(43)											
yes	28	49	24	55	8											
	(49)	(64)	(53)	(71)	(57)											
Corneal edema						0.4614	0.1824	0.4321	0.7663	0.7846	0.6983	0.0958	0.1701	0.2925	0.888	0.9282
	54	77	44	72	13											
no	(95)	(99)	(98)	(94)	(93)											
yes	3 (5)	1 (1)	1 (2)	5 (6)	1 (7)											
Keratic precipitate						**0.0400**	0.2507	0.1627	0.2127	0.2494	0.6902	**0.0107**	0.6630	**0.0072**	0.8694	**0.035**
	12	23	15	10	5											
no	(21)	(30)	(33)	(13)	(36)											
yes	45 (79)	54 (70)	30 (67)	67 (87)	9 (64)											
Character of keratic						0.9713	0.52	0.8824	0.8824	0.507	0.8858	0.8858	0.7752	>0.9999	0.6360	0.6360
precipitates	14	41	22	28	9											
not	(25)	(53)	(49)	(36)	(64)											
documented	16	18	10	26	3											
	(28)	(23)	(22)	(34)	(21)											
granulomatous	21 (37)	14 (18)	10 (22)	19 (25)	2 (14)											
Fine	6	4 (5)	3 (7)	4	0 (0)											
Pigmented	(11)			(5)												
Location of keratic precipitate	14 (25)	38 (49)	37 (82)	40 (52)	5 (36)	0.6053	0.2713	0.8841	0.8913	0.3416	0.2798	0.2267	0.47459	0.3226	0.3226	0.3072
not documented	14 (25)	11 (14)	11 (24)	15 (19)	2 (14)											
Arlt´s triangle	25 (44)	28 (36)	29 (64)	22 (29)	7 (50)											
diffuse scattered	2 (4)	0 (0)														
endotheliitis			0 (0)	0 (0)	0 (0)											
Cells in anterior chamber	29 (50)	48 (62)	28 (62)	28 (36)	7 (50)	0.5991	0.1846	0.2519	0.2519	0.9531	0.9899	0.9899	0.3852	>0.999	0.4162	0.4162
no	28	29	17	17	7											
yes	(49)	(38)	(38)	(38)	(50)											
Hypopyon																
no	56	77	43	77	14	0.2434	0.1554	0.4246	0.2434	0.6177	0.0621			0.0621		
yes	(98)	(100)	(95)	(100)	(100)											
	1 (2)	0 (0)	2 (4)	0 (0)	0 (0)											
Fibrin						0.2145	0.2640	0.5834	0.0898	0.381	0.1263	0.4976	0.8949	**0.0435**	0.2008	0.8096
no	53	67	43	64	12											
yes	(93)	(87)	(96)	(83)	(86)											
	4 (7)	10 (13)	2 (4)	13 (17)	2 (14)											
Iris Synechiae																
no	57	68	42	77	13	**0.0043**	**0.0075**	**0.0479**		**0.0421**	0.3688	**0.0285**	0.6169		0.9506	0.3809
Yes	(100)	(88)	(93)	(100)	(93)											
	0 (0)	9 (12)	3 (7)	0 (0)	1 (7)											
Irisatrophy																
no	55	71	43	68	12	0.3293	0.3008	0.809	0.0881	0.1171	0.4711	0.4149	0.43 ns	0.1777	0.2008	0.7839
yes	(96)	(92)	(96)	(88)	(86)											
	2 (4)	6 (8)	2 (4)	9 (12)	2 (14)											
Iris																
heterochromia	57	76	44	31	14	<**0.0001**	0.3878	0.258	<**0.0001**		0.6983	<**0.0001**	0.6681	<**0.0001**	0.5737	<**0.0001**
	(100)	(99)	(98)	(40)	(100)											
no		1	1	46												
yes	0 (0)	(1)	(2)	(60)	0 (0)											
Vitreous haze																
no	55	69	31	30	10	<**0.0001**	0.1886	0.0001	<**0.0001**	**0.0025**	**0.0041**	<**0.0001**	0.0644	**0.0014**	0.857	**0.0244**
yes	(96)	(90)	(69)	(39)	(71)											
	2 (4)	8 (10)	8 (31)	47 (61)	4 (19)											

(*Continued*)

Table 2. (Continued)

						P Value										
	CMV N = 57	**HSV N = 77**	**VZV N = 45**	**RV N = 77**	**MV N = 14**		**CMV vs HSV**	**CMV vs VZV**	**CMV vs RV**	**CMV vs MV**	**HSV vs VZV**	**HSV vs RV**	**HSV vs MV**	**VZV vs RV**	**VZV vs MV**	**RV vs. MV**
Macular edema	54	75	40	77	13	**0.0414**	0.4208	0.2754	0.0417	0.7846	0.0511	0.1546	0.3809	**0.0028**	0.6679	0.0184
no	(95)	(94)	(89)	(100)	(93)											
yes	3 (5)	2 (6)	5 (11)	0 (0)	1 (7)											
Lens																
Phakic	38	37	15	9	4	<**0.0001**	0.154	**0.0028**	<**0.0001**	**0.008**	0.884	<**0.0001**	**0.0429**	<**0.0001**	0.6083	**0.0002**
	(67)	(48)	(33)	(12)	(29)											
Corticonuclear cataract	11 (19)	23 (30)	14 (31)	55 (71)	3 (21)											
Posterior subcapsular	0 (0)	3 (4)	0 (0)	5 (6)	0 (0)											
cataract	8	14	16	8	7 (50)											
Pseudophakic	(14)	(18)	(36)	(10)												

CMV = cytomegalovirus, GWC = Goldmann–Witmer coefficient, HSV = herpes simplex virus, IOP = Intraocular pressure, MV = multiple virus, RV = rubella virus, VZV = varicella-zoster virus

not documented data excluded

Kruskal-Wallis test, Chi-square-Test, Mann-Whitney Test were performed.

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(S1 Table; S1 Fig). IL-12, IL-15, Eotaxin, IP-10, MCP-1, MIP-1b, and VEGF showed significant differences between CMV, HSV, and RV (P<0.005) (S1 Fig). In a further analysis, we excluded younger (<30 years) and older (>73 years) patients in each cohort to rule out the age specificity and have similar gender distribution (CMV: 16/23, 7 female/9 male; HSV: 23/34, 11 female/12 male, VZV: 7/16, 6 female /1 male, RV: 59/77, 22 female/ 37 male). Thereafter, no significant values were found in the Kruskal-Wallis-test, except of increased IP-10 in CMV compared to VZV patients (P = 0.0138). Also, Spearman correlations showed no significances between immune mediators and the age-matched group of all cohorts. However, significant differences were measured on the above-mentioned immune mediators (IL-12: P = 0.0106, IL-15: P = 0.0109, Eotaxin: P = 0.0122, IP-10: P = 0.012, MCP-1: P = 0.013, MIP-1b: P = 0.0106; VEGF: P = 0.0106) between female and male in CMV patients in the age-matched group. These results could be confirmed in the Spearman´s test (r = 0.2372; P<0.005). For all patients in the CMV cohort, significant differences of other immune mediators between female and male were also measured (IL-1RA: P = 0.0338; IL-5: 0.025; IL-9: P = 0.0146; IL-10: P = 0.0006; IL-13: 0.0088; FGFbasic: P = 0.0212; IFN-g: P00.0041; PDGF: P = 0.0043; RANTES: P = 0.267), whereby the women showed the lowest levels. Other VAU cohorts did not show any gender differences. In the CMV cohort, IL-10 (r = -0.3439, P = 0.0292), IFN-g (r = -0.448, P = 0.0321), MCP-1 (r = -0.4558, P = 0.0288), and MIP1a (r = -0.5088, P = 0.0132) were found to be negatively correlated with IOP values. Seven patients were considered treatment "naïve" meaning without receiving glaucoma treatment–which did not negatively confound the correlation since the patients were presented with low IOP values. CMV patients receiving acetazolamide (n = 10) revealed significantly lower immune mediator levels which we had already reported in our previous work.[10]

Discussion

Our data demonstrate the epidemiology, the clinical characteristics, the development of glaucoma and surgical interventions, and the distribution of immune mediators in AH in immunocompetent patients with VAU of different entities. Out of the total 270 patients, one third were tested for HSV and one third for RV infection followed by CMV and VZV. In the

Table 3. Demographic parameters of the glaucoma cohort.

N		52
Age (years)		48.0±18.8
Gender (male/female)		36 (69.2%) / 16 (30.8%)
Pathogenic virus	RV	11 (21.2%) (AI: 24.31±45.71)
	CMV	32 (61.5%) (AI: 30.07±81.57)
	HSV	5 (9.6%) (AI: 5.24±2.32)
	VZV	3 (5.8%) (AI:3.15±0.13)
	MV	1 (1.9%) (AI: 2.99)
Before AC stab incision	Side (right/left)	31 (59.6%) / 21 (40.4%)
	Involvement (unilateral/bilateral)	50 (96.2%) / 2 (3.8%)
	Course of disease (acute/chronic)	38 (73.1%) / 14 (26.9%)
	Previous keratitis	9 (17.3%)
	Visual acuity (logMAR)	0.16±0.32
	IOP	29.79±10.66 mmHg
	Local therapy	2.08±0.81
	Systemic glaucoma therapy (acetazolamid)	18 (34.6%)
	Steroid therapy (local)	37 (71.2%)
Clinical findings	Conjunctival redness	28 (53.8%)
	Corneal edema	1 (1.9%)
	Keratic precipitates	42 (80.8%)
	Character of precipitates (granulomatous/fine/pigmented /not documented/nothing)	8 (15.4%) / 22 (42.3%) / 7 (13.5%) / 6 (11.5%) / 9 (17.3%)
	Location of precipitates (Arlt´s triangle/diffuse scattered/ endotheliitis/not documented)	8 (15.4%) / 27 (51.9%) / 2 (3.8%) / 15 (28.8%)
	Cells in anterior chamber	18 (34.6%)
	Hypopyon	0 (0%)
	Fibrin	4 (7.7%)
	Iris Synechiae	2 (3.8%)
	Irisatrophy	6 (11.5%)
	Heterochromia	8 (15.4%)
	Lens (corticonuclear cataract or subcapsular posterior cataract/ pseudophacic/ clear lens	14 (26.9%) /11 (21.2%) / 27 (51.9%)
	Vitreous Haze (0/+1/+2/+3)	47 (90.4%) / 4 (7.7%) / 1 (1.9%) / 0 (0%)
	Retinal infiltrates	0 (0%)
	Macula edema	0 (0%)
Recurrence	recurrence (no /yes /not documented)	13 (25.1%) / 37 (71.1%) / 2 (3.8%)

AC = anterior chamber, CMV = cytomegalovirus, HSV = herpes simplex virus, MV = multiple virus, RV = rubella, VZV = varicella-zoster virus, IOP = intraocular pressure

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glaucoma cohort, however, 61% of all patients had positive results for CMV, followed by RV infection (21%). Surprisingly, we measured more than two virus antibodies in 14 eyes and classified this group as MV which has not been shown yet. The range consisted of 28 years to 81 years in the MV group.

Clinical findings of virus-associated anterior uveitis

CMV and RV AU occur predominantly in younger and male patients, while VZV and HSV AU appeared mainly in elderly patients with a predominance in females. These results are

Table 4. Descriptive statistics of the glaucoma subgroup receiving a surgical intervention.

		Glaucoma 1st surgery (n = 27)			Glaucoma 2nd surgery (n = 10)	
		MIGS (n = 16)	TE (n = 9)	CPC (n = 2)	Trabectome (n = 2)	TE (n = 8)
Age (years)		62.8±12.4	52.1±19.7	52.5±31.8	60.5±13.4	55.9±12.8
Gender (male/ female)		16 (100%)/0 (0%)	8 (88.9%)/ 1 (11.1%)	2 (100%)/0 (0%)	2 (100%)/ 0 (0%)	8 (100%)/0 (0%)
Side (right/ left)		9 (56.2%)/7 (43.8%)	5 (55.6%)/4 (44.4%)	1 (50.0%)/1 (50.0%)	0 (0%)/2 (100%)	5 (62.5%)/3 (37.5%)
Virus	RV	7 (43.8%)	4 (44.4%)	0 (0%)	1 (50.0%)	3 (37.5%)
	CMV	4 (25.0%)	2 (22.2%)	0 (0%)	1 (50.0%)	2 (25.0%)
	HSV	3 (18.8%)	1 (11.1%)	1 (50.0%)	0 (0%)	1 (12.5%)
	VZV	1 (6.3%)	1 (11.1%)	1 (50.0%)	0 (0%)	2 (25.0%)
	MV	1 (6.3%)	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)
pre-Op VA (logMAR)		0.21±0.38	0.75±0.20	0.30±0.71	0.70±0.14	0.40±0.36
pre-Op IOP (mmHg)		28.40±9.63	28.00±9.96	27.00±8.49	30.50±13.43	27.75±9.48
Number of glaucoma medications		1.88±0.89	2.00±1.00	2.00±0.00	2.00 ± 0.00	2.25±1.04

CPC = cyclophotocoagulation, CMV = cytomegalovirus, HSV = herpes simplex virus, MIGS = minimal-invasive glaucoma surgery, MV = multiple virus, TE = trabeculectomy, VA = visual acuity, VZV = varicella-zoster virus

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concordant to the literature.[17–20] Interestingly, studies report that CMV tends to more commonly affect Asian populations and it is not uncommon in immunocompetent patients.[36,37] Furthermore, it may present as a recurrent acute or chronic inflammation, resembling PSS, herpetic AU, or FUS in Asia.[36] Thus, Asian patients commonly present chronic CMV AU as FUS, while many studies in Europe confirmed that FUS is almost always related to RV [5,36,38] and PSS is associated with CMV[10,39,40] which is characteristically accompanied

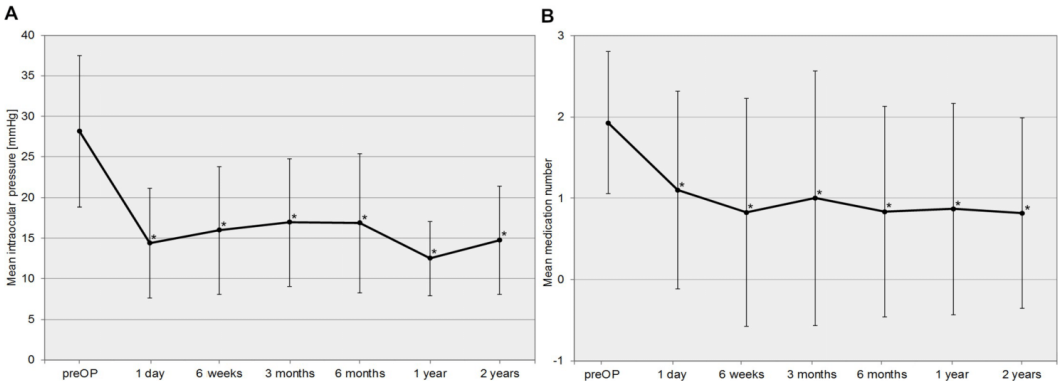

Fig 2. **a** Trendline chart of the intraocular pressure (IOP) comparing preoperative to postoperative follow-up data. **b** Trendline chart of the number of glaucoma medications comparing preoperative to postoperative follow-up data.

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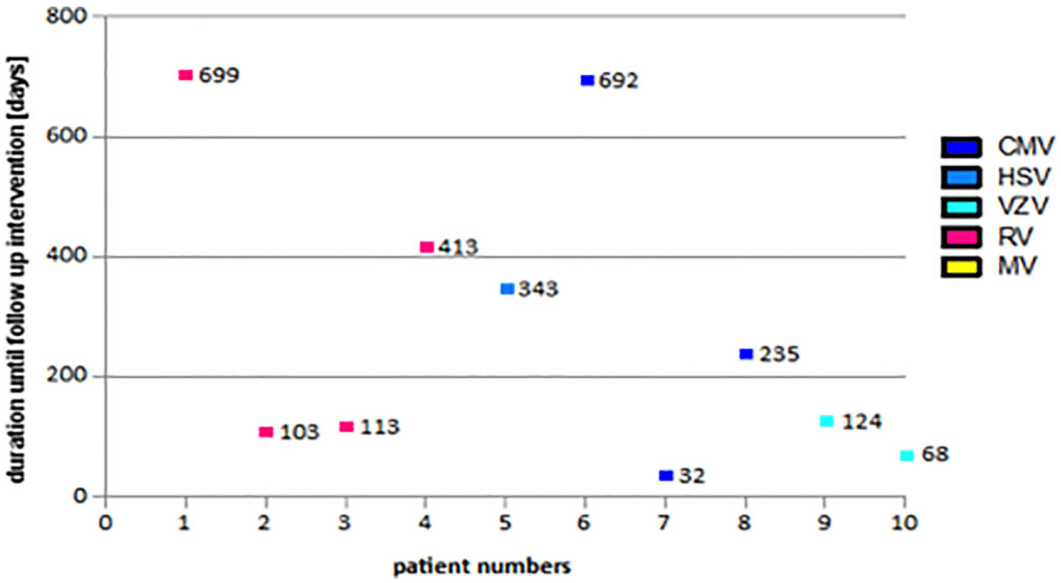

Fig 3. Duration of follow-up interventions in glaucoma patients (CMV = cytomegalovirus, HSV = herpes simplex virus, MV = multiple virus, RV = rubella virus, VZV = varicella-zoster virus).

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with acute IOP spikes. These IOP elevations can make acute glaucoma surgery necessary or end in developing a chronic course of the disease. Our data revealed that CMV is also not as uncommon in the European population, particularly as an acute uveitis (96%) while RV takes on a chronic phenotype (84%) which we confirmed in the immune mediator analysis in our prework.[10] The clinical parameters in CMV show concordance with the reports from previous studies; laterality is almost always unilateral, and endotheliitis and/or corneal edema can manifest due to IOP elevation. In contrast, HSV AU usually follows an acute recurrent course, is typically unilateral, presents KPs, and elevated IOP. In addition, many HSV patients show a chronic course with a diagnosed keratitis, although HSV AU can also occur in the absence of corneal involvement.[19,20] Our data align with comparative research.[19,20] We also observed iris synechia and iris atrophy in some patients which is more common in HSV AU.[18,41,42] A bilateral manifestation of HSV infections is a challenging diagnosis (14% of our cohort) and the focus is on avoiding misdiagnosis as non-infectious uveitis.[43] Frequently high IOPs >30 mmHg were observed in 46 to 90% of patients while in our cohort only 4% of HSV patients were affected.[17,18,20] Only 9.6% of the glaucoma cohort were associated with HSV. The outlined research data, however, presented only few patients, limited to 8 to 39 individuals, in contrast to our patient cohorts.

Furthermore, we can present a large study cohort of VZV cases (45 patients) with confirmed VZV virus in AH. This number has not been presented in the literature to the best of our knowledge. In general, the incidence of VZV increases with age, especially patients above the age of 60 years. VZV AU is commonly associated with herpes zoster ophthalmicus with or without skin rash affecting the distribution of the ophthalmic nerve. Surprisingly, our VZV

Table 5. Correlation of antibody index of Goldmann-Witmer coefficient and glaucoma parameters.

		RV		CMV		HSV		VZV		MV	
		AI	p	AI	p	AI	p	AI	p	AI	p
IOP before AC stab incision	IOP≤21 mmHg	21.6 ±41.18 (N = 56)	0.180	28.4 ±42.3 (N = 18)	0.943	9.07 ±13.7 (N = 60)	0.793	7.16±9.9 (N = 30)	0.373	4.3±1.7 (N = 10)	0.22
	IOP≥22 mmHg	45.7 ±73.8 (N = 20)		27.1 ±74.6 (N = 38)		10.2 ±13.06 (von N = 13)		13.6 ±26.4 (von N = 15)		2.98±2.7 (N = 3)	
IOP before AC stab incision	IOP≤30 mmHg	21.3 ±42.9 (von N = 70)	0.066	22.7 ±35.3 (N = 32)	0.697	9.4±13.7 (N = 71)	0.476	9.7±18.7 (N = 37)	0.738	4.3±1.7 (N = 10)	0.57
	IOP≥31 mmHg	104.8 ±89.3 (N = 6)		33.99 ±92.3 (N = 24)		4.56±2.6 (von N = 2)		7.4±7.6 (von N = 8)		5.98±2.7 (N = 3)	
Topical glaucoma therapy	no	32.2 ±57.4 (N = 45)	0.293	31.1 ±51.4 (N = 10)	0.889	8.3±7.06 (N = 35)	0.881	11.6 ±21.6 (N = 27)	0.175	3.6±1.1 (N = 5)	0.28
	yes	21.7 ±44.0 (N = 31)		26.7 ±68.7 (N = 46)		10.16 ±17.5 (von N = 38)		5.79 ±5.32 (von N = 18)		5.36±2.2 (N = 8)	
Systemic glaucoma therapy (acetazolamid)	no	26.2 ±49.9 (N = 70)	0.665	34.4 ±82.9 (N = 34)	0.211	9.58 ±13.9 (N = 68)	0.437	8.5±17.6 (N = 35)	0.563	3.82 ±0.89 (N = 9)	0.14
	yes	47.8 ±78.7 (N = 6)		16.8 ±16.02 (N = 22)		4.97 ±2.02 (von N = 5)		11.99 ±16.4 (N = 10)		6.64 ±2.59 (N = 4)	
Glaucoma surgery	no	28.54 ±53.6 (N = 65)	0.319	29.6 ±69.0 (N = 50)	0.268	9.56 ±13.9 (N = 68)	0.679	9.59 ±17.6 (von N = 43)	0.068	4.51 ±1.78 (N = 11)	0.98
	yes	24.3 ±45.7 (N = 11)		9.8 ±10.99 (N = 6)		5.23 ±2.32 (N = 5)		3.15 ±0.13 (N = 3)		5.68±3.8 (N = 2)	

AC = anterior chamber, AI = antibody index, CMV = cytomegalovirus, HSV = herpes simplex virus, IOP = intraocular pressure, VZV = varicella-zoster virus

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cohort did not reveal any skin lesions in the past and all patients were immunocompetent. VZV patients followed an acute or chronic course in an equal distribution, laterality was in most cases unilateral, but also bilateral. Although HSV and VZV are clinically similar, the elevated IOP was more common in VZV than in HSV and the vitritis was more prominent in VZV than in HSV patients. In contrast, our RV patients showed vitreous haze in two-thirds of cases. To note, RV AU has a wider spectrum of clinical findings than the clinical features typical of FUS.[44] FUS is a clinical syndrome which is associated with RV. A recent study of Groen-Hakan et al. confirmed that RV AU and FUS are not exchangeable.[44] However, the combination of AU and vitreous haze can cause a dilemma based on suggestion of the diagnosis of intermediate uveitis. Moreover, a study showed that 98% of FUS patients demonstrated disc hyperfluorescence on fluorescence angiography.[45] Therefore, a confirmation of RV infection in AH is meaningful to ensure the correct diagnosis. Unnecessary administration of corticosteroids prevents further complications such as cataract and glaucoma development.

Interestingly, the MV cohort showed the highest mean age compared to all other cohorts and two-third were male patients. To our knowledge, a cohort with multiple tested viruses was not mentioned in current literature. This is most likely because not all viruses were tested simultaneously in comparative research. We should be aware that older patients in particular might show coinfections with significant AI titers for different and MV due to disruption of the blood-aqueous humor barrier–which might play a crucial role in therapeutic decision and visual outcome of the patients. Interestingly, no previous publication showed positive results for more than one of these viruses. Only a recent study measured a positive GWC for MV in five patients, but they were negative in PCR for all investigated agents.[44] However, it was not further discussed.

Glaucoma

The most common and known complication of VAU is the development of secondary glaucoma.[46] Dick et al. found that uveitis caused a significantly higher 5-year risk of glaucoma (20% vs. 9%) and severe consequences for visual acuity.[47] Several studies reported that secondary glaucoma develops in 10–40% of VAU subjects during the course of the disease[16–20,46] which is in line with our data. Prevalence of secondary glaucoma was similar in RV, HSV, and VZV associated uveitis (p = 0.686).[18] CMV showed very high IOP values during active inflammatory episodes in comparison to RV, HSV, and VZV.[10,11,15,36] Exceptional cases in uveitis have always been reported and have to be considered in therapeutic decisions. In our study cohort, glaucoma surgery was necessary in approximately 10% of all VAU participants. Considering the virus antigen distribution in our study cohort, CMV and rubella virus associated uveitis were on risk for developing glaucoma and needing glaucoma surgery. In a recently published paper, analyzing CMV AU surgery of uncontrolled IOP was necessary in up to 25.7% of the study cohort.[48] In RV AU, no comparative literature considering different options in glaucoma surgery can be found. Our treatment regime of antiviral and glaucoma therapy can be seen as playing a fundamental role for the successful survival of our study cohort leading to an adequate number of cases needing glaucoma surgery. Protective factors in preventing first-line surgery are early intravenous or oral antiviral medication.[47,48]

MIGS–only an exceptional use in VAU

Regarding different glaucoma surgeries, MIGS showed a reliable IOP reduction in the short-term period and then started to fail in 56% of all MIGS cases (9 out of 16) over the course of two years. The great advantage of MIGS can be found in its low complication level.[11,27–30] Thus, this surgical principle was promising in VAU eyes to avoid inflammatory recurrence due to the intra- and perioperative interventions needed in filtrating procedures. Glaucoma surgery, especially in young patients with IOP >30mmHG, remains challenging. Avoiding vision-threatening complications such as choroidal effusion, bleeding, and postoperative hypotony need to be the primary goal of a surgeon. Thus, different MIGS devices were used in our VAU cohort as first-step procedure after gaining knowledge of efficiency in primary open angle glaucoma (POAG) and exfoliative glaucoma. According to our retrospective data, we can state that MIGS can be used in secondary glaucoma, however, the device should be carefully chosen for the individual needs of a patient by specialized glaucoma and uveitis experts. MIGS might work (approximately 50% of our MIGS cohort) in individual secondary glaucoma cases but there are no data showing evidence for building up guidelines to generally start with MIGS. The size of our glaucoma surgery cohort (n = 27) does not allow statistically relevant conclusions about differences of MIGS devices.

There exists one comparative study showing a significant reduction of IOP 40±10 mmHg (range 33–58 mmHg to 13±1 mmHg) and glaucoma medication (decrease of 2.3 number of medications) in Trabectome® surgery in CMV AU, however, it only considers a one-year period of time.[11] Shimizu et al. reported on filtrating and Trabectome® surgery in different uveitis cohorts including VAU and found a higher survival rate of trabeculectomy (83%) compared to MIGS (75%).[16] They reported a higher risk of surgical failure in young male patients with nongranulomatous uveitis and prolonged postoperative inflammation.[16] Thus, trabeculectomy as a filtrating procedure remains the widely accepted standard in VAU to achieve an effective IOP reduction. This study is the first, to the best of our knowledge, to analyze the outcome of MIGS and filtrating procedures in different viral types of a large VAU cohort.

High-failure rate of glaucoma surgery in VAU

In comparing the results of VAU secondary glaucoma to POAG data, we would have to report about ineffective surgical procedures considering a failure rate of 37% of the complete cohort. Iwao et al. found a significantly higher 3-year success rate of 89.7% in POAG compared to 71.3% in uveitic glaucoma.[49] In our cohort, 10 patients received a second intervention due to surgical failure and uncontrolled individual pressure (Trabectome® n = 4; iStent inject® n = 3; canaloplasties ab externo n = 2, TE+ Mitomycin C n = 1). These results imply a failure rate of 11.1% in trabeculectomy (1/9), 50% in Trabectome® (4/8), 50% in iStent inject® (3/6) and 100% in canaloplasties (2/2). These data cannot be considered as statistically significant due to low patient numbers. Instead, it should only be used to highlight a trend. Interpretation of these data does not allow conclusions about IOP reduction between single MIGS procedures. It is of great importance to choose the right MIGS device to achieve a long-lasting IOP reduction. MIGS can be recommended to start with in young patients and patients with high IOP in order to reduce intra- and perioperative risks of vision loss.

Considering the trabeculectomy surgery, our cohort showed a low-failure rate in contrast to comparative research. Kwon et al. reports a failure rate of as high as 51.9% in trabeculectomies.[50] Shimizu et al. stated data of failure in filtrating surgery and Trabectome® in 21.3% of all cases (n = 10 out of 47).[16] Additionally, a 25% failure rate of trabeculectomy was found by Ceballos et al.[51] These results align more accurately with our results regarding the filtrating surgery.

Interestingly, VZV showed a failure rate in the first three months after the first surgical intervention, while in RV and CMV AU, a high mean variation was observed. The informative value is limited by the small number of subjects. To the best of our knowledge, no comparative data of VZV and RV were found in literature. A significant risk factor of failure lies in the postoperative relapse of inflammation. Thus, a controlled inflammatory situation pre- and postoperative is crucial for therapeutic success.[16,52] In addition, hypotony is one of the most feared complications following glaucoma surgery in uveitis. In our study cohort, there were no cases of hypotony to report. Kwon et al. reported early hypotony rates of trabeculectomy and Ahmed valve implantation at 30%, as well as late hypotony at 11–15%.[50] In contrast, the research group of Iwao et al. found no significant difference in the frequency of highly feared surgical complications such as bleb leakage, hypotensive maculopathy, hemorrhage, and endophthalmitis.[49] This is in line with our data. In summary, the most important decision in minimalizing failure risk is to obtain a stable intraocular inflammatory situation including antiviral and glaucoma medication before starting surgical interventions, and if possible, at intervals of at least three months of the last acute inflammation.[47] Patients, however, have to

be informed about the complication profile and close postoperative follow-up appointments in different viral VAU types. [33–35,46,49,52]

Antibody index and immune mediators

The question of whether the severity of VAU can be measured by AI or immune mediators remains relevant. We observed a higher AI in CMV and RV in patients with IOP over 30mmHg compared to other patients with moderate IOP. We assume that there might be a relation between AI, inflammatory process, and glaucoma development.[11] Interestingly, we measured a negative correlation between mediators and IOP in CMV AU which is published in our previous work.[10] IOP elevation might be due to several parameters: a trabecular meshwork obstruction and a decreased outflow of AH, a reduction of AH drainage from the anterior chamber and degradation of mediators, or apoptosis. These data focused on all immune mediators in all groups which showed a similar distribution. Surprisingly, we observed that women showed the lowest immune mediators´ levels in the CMV cohort. Overall, it is difficult to state a clear judgement about the immune mediators. Therefore, prospective studies need to be conducted to follow-up on these trends.

Limitations

This study has several limitations. Its retrospective nature leads to lack of information on the clinical findings such as type and distribution of KPs. Because we are a tertiary referral center, we sometimes see the patients very late in a chronic status and the onset of the disease could not be established. After confirmation of diagnosis, some patients continued their follow-up with the first center. In contrast, the glaucoma cohort showed a close follow-up. Normally, a correct diagnosis was made by the analysis of the AH and a PCR was omitted. There was not enough AH left in all cases to perform the immune mediator analysis. Clinical data and experience have always been one of the most important features in diagnosis and therapy of uveitis. Therefore, although retrospective assessed, our data of this large number of VAU patients are still valuable information for the outpatient clinicians. Due to the retrospective study design and that data have been assessed starting in 2009, retinal nerve fiber layer (RNFL) was not analyzed or available in all patients of the study group. The significance of this information as glaucoma parameter in uveitis was contained by Moore et al.[53] Their study group reported an increased RNFL thickness than anticipated in secondary glaucoma.[53] Furthermore, we did not analyze MIGS with combined cataract surgery, thus this could be an idea to follow-up on when IOP elevation might be due to a severe cataract and inflammation.

Prospective studies, including repeated AH analysis over time, need to be conducted to evaluate a correlation between inflammation and the rise of cytokine levels in comparison to glaucoma development, progression, and necessary surgical treatment.

Conclusion

There are several different clinical characteristics which describe the individual virus entities. In some cases, however, the clinical findings present similarities which makes a determination of the correct virus uncertain. Elderly male patients in particular could present MV simultaneously. Therefore, it is worthwhile to analyze antibody synthesis in AH to establish the appropriate treatment at an early stage of disease. More than half of our patients already had a chronic course of VAU and were not yet set with adequate therapy. During this process, cataract and glaucoma may develop as a vision-threatening complication of uveitis. In particular, the glaucoma therapy and surgical interventions remain challenging in VAU and need to be conducted by specialized centers. In our study, we were able to show a significant IOP

reduction and thus a controlled glaucoma situation by filtrating surgery. Additionally, MIGS can certainly be used as first-line treatment in individual cases of VAU, such as young age and high preoperative IOP > 30mmHG to avoid choroidal effusion and hypotony. At this time, virus association for the outcome of glaucoma surgery cannot be stated because of the low total number of patients needing surgical interventions.

Supporting information

S1 Table. Summary of immune mediators' levels (pg/mL) in log10.
(DOCX)

S1 Fig. Immune mediators' distribution in four study cohorts.
(TIFF)

S1 Data.
(XLSX)

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Author Contributions

Conceptualization: Dominika Pohlmann.

Data curation: Dominika Pohlmann, Milena Pahlitzsch, Sylvia Metzner, Matthias Lenglinger.

Formal analysis: Dominika Pohlmann, Milena Pahlitzsch.

Investigation: Dominika Pohlmann, Stephan Schlickeiser.

Methodology: Dominika Pohlmann, Milena Pahlitzsch, Stephan Schlickeiser.

Project administration: Dominika Pohlmann.

Resources: Stephan Schlickeiser.

Software: Stephan Schlickeiser.

Supervision: Stephan Schlickeiser, Uwe Pleyer.

Validation: Dominika Pohlmann, Milena Pahlitzsch.

Visualization: Dominika Pohlmann, Milena Pahlitzsch, Anna-Karina B. Maier, Sibylle Winterhalter, Uwe Pleyer.

Writing – original draft: Dominika Pohlmann, Milena Pahlitzsch.

Writing – review & editing: Stephan Schlickeiser, Eckart Bertelmann, Sibylle Winterhalter, Uwe Pleyer.

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2.5. Neuroprotektive Therapieansätze durch die topische Anwendung von Coenzym Q10

Davis BM, Tian K (shared first), Pahlitzsch M, Brenton J, Ravindran N, Butt G, Malaguarnera G, Normando EM, Guo L, Cordeiro MF. Topical Coenzyme Q10 demonstrates mitochondrial-mediated neuroprotection in a rodent model of ocular hypertension. Mitochondrion. 2017 May 24. pii: S1567-7249(17)30142-3. doi: 10.1016/j.mito.2017.05.010. [Epub ahead of print]

Neben den chirurgischen Verfahren und ihren Fortschritten gibt es neuroprotektive Therapieansätze in der Behandlung neurodegenerativer Erkrankungen. Coenzym Q10 (CoQ10) gilt es vielversprechender Wirkstoff. CoQ10 ist ein Antioxidans mit bekannter neuroprotektiver Aktivität. In gebundener Form (Vitamin E Derivat) zeigt es als topische Lösung eine neuroprotektive Wirkung mit guter Bioverfügbarkeit. Dies wurde in Vorläuferstuden in vitro in unterschiedlichen Zelllinien der retinalen Ganglionzelle bestätigt.
In dieser Studie wird ein adultes okuläres Hypertensionsmodell an Dark-Agouti-Ratten genutzt, die über den Zeitraum von drei Wochen zweimal täglich mit CoQ10/TPGS-Mizellen oder TPGS-Vehikeln behandelt werden. Am Studienendpunkt wird in vivo mittels DARC Technologie (Detektion apoptotischer retinaler Ganglienzellen) die Apoptose der RGC erfasst und mit der Kontrollgruppe verglichen.
Die CoQ10/TPGS Lösung zeigt einen signifikanten neuroprotektiven Effekt im Vergleich zu der Kontrollgruppe in vivo mitels DARC ($p<0,05$) und ex vivo in der histologischen Kontrolle ($p <0,01$).

Der nachfolgende Text entspricht dem Abstrakt des genannten Papers:

Originalsprachiges Abstrakt

„Coenzyme Q10 (CoQ10) is a mitochondrial-targeted antioxidant with known neuroprotective activity. Its ocular effects when co-solubilised with α-tocopherol polyethylene glycol succinate (TPGS) were evaluated. In vitro studies confirmed that CoQ10 was significantly protective in different retinal ganglion cell (RGC) models. In vivo studies in Adult Dark Agouti (DA) rats with unilateral surgically-induced ocular hypertension (OHT) treated with either CoQ10/TPGS micelles or TPGS vehicle twice daily for three weeks were performed, following which retinal cell health was assessed in vivo using DARC (Detection of Apoptotic Retinal Cells) and post-mortem with Brn3a histological assessment on whole retinal mounts. CoQ10/TPGS showed a

significant neuroprotective effect compared to control with DARC ($p<0.05$) and Brn3 ($p<0.01$). Topical CoQ10 appears an effective therapy preventing RGC apoptosis and loss in glaucoma-related models."

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Topical Coenzyme Q10 demonstrates mitochondrial-mediated neuroprotection in a rodent model of ocular hypertension

Benjamin Michael Davis[a,1], Kailin Tian[a,1], Milena Pahlitzsch[a], Jonathan Brenton[a], Nivedita Ravindran[a], Gibran Butt[a], Giulia Malaguarnera[a], Eduardo M. Normando[a,b], Li Guo[a], M. Francesca Cordeiro[a,b,*]

[a] Department of Visual Neuroscience, UCL Institute of Ophthalmology, London EC1V 9EL, United Kingdom
[b] Western Eye Hospital, Imperial College London, United Kingdom

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ABSTRACT

Coenzyme Q10 (CoQ10) is a mitochondrial-targeted antioxidant with known neuroprotective activity. Its ocular effects when co-solubilised with α–tocopherol polyethylene glycol succinate (TPGS) were evaluated. *In vitro* studies confirmed that CoQ10 was significantly protective in different retinal ganglion cell (RGC) models. *In vivo* studies in Adult Dark Agouti (DA) rats with unilateral surgically-induced ocular hypertension (OHT) treated with either CoQ10/TPGS micelles or TPGS vehicle twice daily for three weeks were performed, following which retinal cell health was assessed *in vivo* using DARC (Detection of Apoptotic Retinal Cells) and post-mortem with Brn3a histological assessment on whole retinal mounts. CoQ10/TPGS showed a significant neuroprotective effect compared to control with DARC ($p < 0.05$) and Brn3 ($p < 0.01$). Topical CoQ10 appears an effective therapy preventing RGC apoptosis and loss in glaucoma-related models.

1. Introduction

Glaucoma is a progressive neurodegenerative eye disorder estimated to affect 60 million people worldwide (Cook and Foster, 2012; Tham et al., 2014). Glaucoma involves the progressive loss of retinal ganglion cells (RGCs) and their axons, which results in visual field abnormalities and ultimately blindness if left untreated (Garcia-Valenzuela et al., 1995; Quigley et al., 1995). Elevated intraocular pressure (IOP) is presently the only modifiable disease risk factor (Weinreb and Khaw, 2004; Lee et al., 2014a). However, recognition of a subset of glaucoma patients who continue to exhibit visual decline despite therapeutically well-controlled IOP has led to the realisation that novel therapeutic paradigms for this condition are urgently required (Resnikoff et al., 2004).

RGC loss in glaucoma is predominantly thought to occur *via* elevated apoptosis (a type of programmed cell death) (Quigley et al., 1995; Cordeiro et al., 2010) which is mainly mitochondrial dysfunction mediated (Lee et al., 2014a; Ju et al., 2008; Park et al., 2011). While the primary site of injury is thought to occur at the site of the RGC axon in the optic nerve, (Quigley et al., 1977; Minckler et al., 1977; Quigley et al., 1981; Knox et al., 2007) the resulting loss of RGCs (primary degeneration) can also lead to the secretion of pro-apoptotic factors resulting in secondary neurodegeneration and the death of neighbouring RGCs (Davis et al., 2016a). Although the exact mechanism of glaucoma progression remains to be elucidated, elevated oxidative stress has been suggested to contribute to glaucoma pathogenesis (Tezel et al., 2005; Yuki et al., 2010). Mitochondria are a source and target of oxidative stress and therefore are key in the development of neuroprotective strategies for RGC preservation in glaucoma (Chrysostomou et al., 2013).

Coenzyme Q10 (CoQ10) is a mitochondrial targeted antioxidant that plays an essential role in the normal function of the electron transport chain. CoQ10 has been reported to exhibit neuroprotective activity in a range of disorders including; cerebral ischemia, (Ahmed et al., 2015) Parkinson's disease and Huntington's disease (Klongpanichapak et al., 2006). In addition to its role as an antioxidant, CoQ10 is also reported to protect against glutamate excitotoxicity *in vivo* through the inhibition of mitochondrial depolarization (Papucci et al., 2003; Lee et al., 2014b).

Concentrations of CoQ10 in the human retina are reported to decline by up to 40% with age (Qu et al., 2009). The poor aqueous solubility (Fato et al., 2010) and low bioavailability of CoQ10, due in part to its interactions with the multi-drug efflux pump P-glycoprotein (P-gp), have limited the development of topically active formulations of

* Corresponding author at: Department of Visual Neuroscience, UCL Institute of Ophthalmology, London EC1V 9EL, United Kingdom.
E-mail address: m.cordeiro@ucl.ac.uk (M.F. Cordeiro).
[1] Authors contributed equally to the work.

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this drug (Hirano and Iseki, 2008). The interaction of CoQ10 with P-gp, expressed in both corneal epithelial cells (Vellonen et al., 2010) and RGCs (Duncan et al., n.d.) suggests that co-administration of CoQ10 with a P-gp inhibitor would likely enhance the topical delivery and pharmacological effects of this drug (Hirano and Iseki, 2008). α-Tocopherol is a form of vitamin E best known for its role as a lipid soluble antioxidant but is well-documented to inhibit P-glycoprotein (P-gp) activity (Wu et al., 2007; Davis et al., 2015). The mechanism of α-Tocopherol mediated P-gp inhibition is poorly understood but has recently been suggested to occur as a result of indirect modulation of the membrane dipole potential (Davis et al., 2015).

Formulation of CoQ10 into micelles using the vitamin E derivative D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS) has previously been reported to deliver micromolar concentrations of CoQ10 to the vitreous in patients 1 h after administration (Fato et al., 2010). The present study sought to investigate the mechanism of α-Tocopherol mediated P-gp inhibition and assess the neuroprotective effects of CoQ10 and TPGS using immortalised and primary mixed retinal cultures (Galvao et al., 2014; McCarthy et al., 2004). Finally, the efficacy of topically applied CoQ10/TPGS micelles was next evaluated *in vivo* using the well-established Morrison's ocular hypertension model (OHT) (Morrison et al., 1997) and *in vivo* DARC (Cordeiro et al., 2017) and Brn3a-RGC immunohistochemistry as endpoints (Galvao et al., 2013; Davis et al., 2016b).

2. Methods

2.1. Cell culture

Both primary murine retinal mixed cultures (pMC) and an immortalised retinal neuronal (RN) cell line (RGC5, a gift from Dr. Neeraj Agarwal, Department of Cell Biology and Genetics, UNT Health Science Centre, Fort Worth, TX) were used. These cells express retinal neuronal proteins Thy-1, Brn3a, and β3 tubulin (Krishnamoorthy et al., 2001; Burugula et al., 2011; Nadal-Nicolás et al., 2009), and are known to be similar to the 661w photoreceptor cell line and RGCs (Al-Ubaidi, 2014; Van Bergen et al., 2009; Krishnamoorthy et al., 2013). RN were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Paisley, UK), supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 100 U/mL penicillin and 100 mg/mL streptomycin. Primary murine (C57BL/6) mixed retinal cultures were isolated from P1 pups and neuronal cells isolated by incubation in a solution containing 10 units of papain/mL, and cultured in DMEM supplemented with 5% fetal bovine serum (Invitrogen, UK), 100 U/mL penicillin, 100 μg/mL of streptomycin and 0.292 mg/mL glutamine (Gibco, UK), 7.5% sterile dH20 and 1.5 mM KCl (Sigma-Aldrich, UK). The medium was changed completely on day 1 and 50% refreshed on day 2. Cells were used for experiments on day 3.

2.2. P-glycoprotein activity assessment

Analysis of P-gp activity was performed as previously described (Ohashi et al., 2006). Briefly, RN cells were seeded at 4000 cells/well in a 96 well plate for 24 h. On the day of the study, cell monolayers were washed before treatment with varying concentrations of TPGS or verapamil hydrochloride (Sigma-Aldrich), a known P-gp inhibitor for 10 min and incubated for 10 min at 37 °C. After this time, cells were incubated with the P-gp substrate calcein-AM (Invitrogen) for 60 min before P-gp activity was measured by quantifying calcein fluorescence using excitation and emission wavelengths of 485 nm and 530 nm respectively (Safire plate reader). Percentage P-gp activity at each concentration of drug was determined using Eq. (1);

$$\text{Pgp activity } (\%) = 100 - \frac{(RFU_{test} - RFU_{BK})}{(RFU_{MAX} - RFU_{BK})} \quad (1)$$

where; RFU_{test} is the fluorescence in the presence of test compound, RFU_{BK} is the fluorescence in the absence of test compounds and RFU_{MAX} is the fluorescence in the presence of 66 μM verapamil which induced maximal P-gp inhibition. EC_{50} values were determined by fitting results to four-parameter dose response curves.

2.3. Dipole potential assessment

RN cultures were seeded at 4000 cells/well in a 96 well plate and permitted to settle for 24 h before washing well before labelling with 0.5 μM of the fluorescent probe di-8-ANEPPs (Invitrogen, from 2 mM stock solution in ethanol) for 1.5 h in phenol-red free DMEM (Sigma-Aldrich) (Davis et al., 2015). After this time the ratiometric di-8-ANEPS fluorescence intensity at excitation of 420/520 nm and emission of 670 nm using a Safire plate reader for each cell population was recorded before and 10 min after cells were treated with varying concentrations of TPGS for 10 min. The change in fluorescence ratio of di-8-ANEPPS indicates a change in the membrane dipole potential on addition of an agent of interest. The dissociation constant (K_d) of the interaction of TPGS for neuronal cells was determined by fitting the change in di-8-ANEPPs fluorescence ratio to a hyperbolic binding equation as described previously (Davis et al., 2010).

2.4. Immunocytochemistry

pMC were fixed in 4% paraformaldehyde for 15 min before washing twice with PBS and permeabilizing in PBS plus 0.1% Tween-20. Cells were blocked with PBS containing 3% bovine serum albumin (BSA, Sigma-Aldrich, UK) for 1 h prior to incubation with primary antibodies overnight at 4 °C (diluted in PBS containing 3% BSA; see Table 1 for details of antibodies used), followed by the appropriate Alexa Fluor 488 nm or 555 nm secondary antibody for a further hour at a 1:1000 dilution (Life technology, UK). Cells were subsequently washed twice with PBS, before addition of 5 μg/mL cell permeable dye Hoechst 33342 (Molecular Probes, Eugene, OR, USA) for 5 min at room temperature prior to visualisation. Then mounted with mowiol (Merck, UK) and were observed under a confocal fluorescence microscope (LSM 700, Carl Zeiss MicroImaging GmbH, Jena, Germany).

2.5. Reverse transcription PCR assay

To test pMC for retinal neuronal marker expression, total RNA was extracted from primary mixed retinal cultures using RNeasy mini kit following manufacturer's specifications (Qiagen, UK). Complementary DNA (cDNA) synthesis was conducted by QuantiTect Reverse Transcription (Qiagen) according to manufacturer's protocol. The PCR reaction was conducted using the GoTaq G2 DNA polymerase kit (Promega, UK). Primers and cycle conditions are summarised Table 2.

2.6. Oxidative cytotoxicity evaluation and cell viability assays

pMC were plated at 30,000 cell/well in 96-well plates for 24 h. After this time cells were treated with either 20 μM CoQ10 with 57 μM TPGS, or 57 μM TPGS only (vehicle control) for 2 h. The molar ratio of CoQ10 and TPGS chosen was the same as that present in the micelle formulation subsequently used *in vivo*. After this time, treatments were removed before application of varying concentrations of cytotoxic

Table 1
Antibodies source and optimized dilutions.

Antibody	Company	Cat.	Host species	Dilution
Brn3a	Abcam	AB81213	Rabbit	1:200
Thy-1	Abcam	AB225	Mouse	1:500
RBPMS	Abcam	AB152101	Rabbit	1:500
γ-synuclein	Abcam	AB55424	Rabbit	1:1200

Table 2
Summary of PCR primers.

Gene	NCBI ref. (Murine mRNA[cDNA])	Primers Forward (5′- > 3′) Reverse(3′- > 5′)	PCR product length	Tm (°C)
Thy-1	NM_009482.3	TGAGGGAAGTTGGACTGTGC CCCTTCCTGCACGGACTTAG	405	60
Brn3a (Pou4f1)	NM_011143.4	CCTCGTCTGAGAAGATCGCC AACAACGCCTACCCAGAGTG	790	60
γ-synuclein	NM_011430.3	CACACTGAATGCCCTGCCTA ACAGCAGCATCTGATTGGTGA	156	60

insults (DMSO or paraquat, Sigma-Aldrich, UK) which were incubated for 24 h (5% CO_2, 37 °C). Cell viability was then assessed using the Alamarblue (Invitrogen, UK) assay according to manufacturer's instructions. Briefly, the Alamarblue solution was added to each well to a final concentration of 10% v/v. Cells were incubated for 4 h at 37 °C before fluorescence was recorded using a Safire plate reader (excitation of 530 nm and emission of 590 nm) and cell viability determined as previously described (Lancaster and Fields, 1996). Results presented are averages of at least three independent experiments.

2.7. Animals

All animal experiments were performed with procedures approved by the U.K. Home Office and in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. For *in vivo* assessment of experiments: in total 20 Adult male Dark Agouti (DA) rats (Harlan Laboratories, UK) weighing 150 to 200 g were housed in an air-conditioned, 21 °C environment with a 12 h light-dark cycle (140–260 lx), where food and water were available *ad libitum*.

2.8. Ocular hypertension model

Ocular hypertension was surgically induced in the left eye of 20 DA rats as described previously (Morrison et al., 1997). Procedures were conducted under general anaesthesia using a mixture of 37.5% Ketamine (Pfizer Animal Heath, Exton, PA), 25% Dormitol (Pfizer Animal Heath, Exton, PA) and 37.5% sterile water, at 2 mL/kg administered intraperitoneally. Briefly, 50 µL of hypertonic saline solution (1.8 M) was injected into the two episcleral veins using a syringe pump (50 µL/min; UMP2; World Precision Instruments, Sarasota, FL, USA). A propylene ring with a 1 mm gap cut from the circumference was placed around the equator to prevent injected saline outflow from other aqueous veins. The IOP from both eyes of each rat was measured at regular intervals using a TonoLab tonometer (Tiolat Oy, Helsinki, Finland) under inhalational anaesthesia (0.4% isoflurane in oxygen). Daily administration of topical CoQ10/TPGS micelles (0.5% w/v TPGS with 0.1% CoQ10 w/v in PBS, pH 7.4) or TPGS only micelles (0.5% w/v TPGS, vehicle control) was performed in DA rats (two 30 µL drops/day 5 min apart at 10 am each day) starting two days prior to model induction and continuing until model termination (21 days post IOP elevation). Animals underwent DARC imaging before sacrifice three weeks after unilateral IOP elevation.

2.9. Detection of apoptotic retinal cells

Fluorescently labelled Annexin A5 (Anx776, (Cordeiro et al., 2017)) was given by intravitreal administration as described previously (5 µL of 0.4 µg/mL) (Cordeiro et al., 2010; Galvao et al., 2013; Guo et al., 2014). *In vivo* DARC imaging was performed using a modified cSLO (Heidelberg Retina Angiograph 2, Heidelberg Engineering, Dossenheim, Germany) (Cordeiro et al., 2004; Maass et al., 2007) and a 55° field of view centred on the optic disc (Cordeiro et al., 2004; Maass et al., 2007). No complications or intraocular side effects associated with topical treatments were recorded.

2.10. Brn3a immunohistochemistry and confocal microscopy

Brn3a labelling of RGCs in retinal whole mounts was completed as described previously (Davis et al., 2016a). Briefly, eyes were enucleated upon sacrifice and fixed in 4% paraformaldehyde at 4 °C overnight before dissecting retinal whole mounts. Whole mounts were stained for the RGC specific nuclear-localised transcription factor Brn3a using an anti-mouse mAb (1:500, Merck Millipore, Darmstadt, Germany) and examined under confocal microscopy (LSM 710, Carl Zeiss MicroImaging GmbH, Jena, Germany). Each retinal whole mount was imaged as a tiled z-stack at ×10 magnification which was used to generate a single plane maximum projection of the RGC layer in each retina for subsequent analysis. Each whole mount image was manually orientated so that the superior retina was towards the top of the image using *in vivo* cSLO imaging of retinal vasculature as a reference. Retinal image acquisition settings were kept constant for all retinas imaged, allowing comparison of Brn3a expression in each experimental group as previously described (Nadal-Nicolás et al., 2012). Automated quantification of Brn3a labelled RGCs in retinal whole mounts was completed as described previously (Davis et al., 2016a). Naïve Brn3a whole retinal counts from DA rats (Fig. 6) was obtained from our previous work (Davis et al., 2016a).

2.11. Statistical analysis

All data were analysed with the Student's *t*-test or ANOVA with posthoc testing using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) as appropriate. Data were presented as means ± SE and $p < 0.05$ was considered significant.

3. Results

3.1. The vitamin E derivative TPGS modulates P-glycoprotein activity and membrane dipole potential over the same concentration range in immortalised neuronal cells

The effect of TPGS on the viability of immortalised RN cells was first established using the AlamarBlue viability assay (Fig. 1A). The IC_{50} of TPGS after 24 h incubation was found to be 259 ± 14 µM with no significant reduction in cell viability observed up to TPGS concentrations of 132 µM. The calcein-AM P-gp activity assay (Fig. 1B) determined the IC_{50} of verapamil as 1.03 ± 0.02 µM which is similar to that reported elsewhere in the literature (Kishimoto et al., 2016). The IC_{50} of TPGS was found to be 2.48 ± 0.06 µM, in agreement with reports in the existing literature that this molecule is a P-gp inhibitor despite not being a direct P-gp substrate (Collnot et al., 2010). Using the same model, the influence of TPGS on the membrane dipole potential was investigated (Fig. 1C). The interaction of TPGS with this neuronal cell line was found to induce a marked decline in the membrane dipole potential in a similar manner to that previously reported for α-tocopherol which fit a hyperbolic binding equation with a dissociation constant of 2.22 ± 0.03 µM. The striking similarity between the IC_{50} of TPGS for P-gp and the effect of TPGS on the membrane dipole potential provide further evidence to support the hypothesis that modulation of membrane dipole potential indirectly modulates P-gp activity.

3.2. Coenzyme Q10 micelles are neuroprotective in vitro against established models of mitochondrial-mediated neurotoxicity in rodent primary mixed retinal cultures

Primary mixed murine retinal cultures were firstly characterised immunohistochemically (Fig. 2A–D) and by mRNA expression using PCR (Fig. 2E). A proportion of mixed retinal cultures were found to

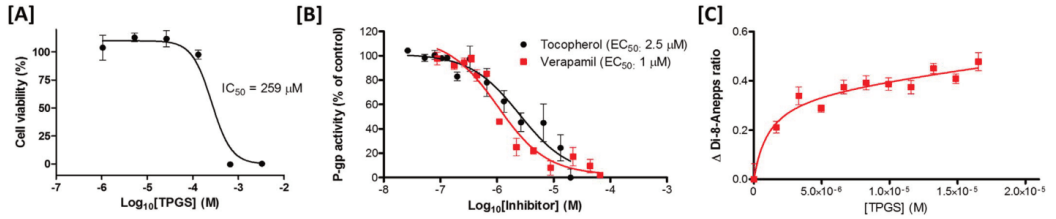


Fig. 1. The P-gp inhibition activity of TPGS in a neuronal cell line closely matches its dipole potential modulating effects [A] Dose response curve (AlamarBlue) for a retinal neuronal cell line after 18 h incubation with TPGS (n = 3). [B] Comparison of the effect of TPGS and verapamil hydrochloride on P-gp activity in the same retinal neuronal cell line. Data expressed as the mean ± SE (n = 6). The figure shows a dose-dependent decrease in P-gp activity with both verapamil hydrochloride and TPGS fit four parameter dose-response curves. [C] Change in membrane dipole potential on titration of TPGS into retinal neuronal cell line as determined by di-8-ANEPPs fit best to a hyperbolic binding equation with a dissociation constant similar to the IC_{50} of TPGS for P-gp (2.48 ± 0.06 μM *versus* 2.22 ± 0.03 μM respectively). Results are means ± SE.

label with the RGC specific markers Brn3a, γ-synuclein, RBPMS and Thy-1 and expression of RGC specific markers was confirmed by PCR (Fig. 2E).

Pre-treatment of pMC cultures with CoQ10/TPGS micelles was found to significantly reduce cell death induced by DMSO and paraquat (unmatched two-way ANOVAs, $p = 0.031$ & $p = 0.002$ respectively) (Fig. 3A–B). Treatment of cells with equivalent concentrations of TPGS alone did not elicit a significant neuroprotective effect in either cytotoxic model.

3.3. *Topically applied Coenzyme Q10 micelles reduce RGC apoptosis in the Morrison's model of ocular hypertension independent of IOP*

Having established the neuroprotective potential of CoQ10/TPGS micelles *in vitro*, we next sought to determine whether topical application of CoQ10/TPGS micelles could induce neuroprotection using a well-established rodent model of experimental glaucoma. Induction of OHT in DA rats resulted in an increase in IOP (Table 3), which peaked 1-day post-surgery in all treatment groups (Fig. 4A) and returned to baseline levels by the three-week time point. No significant change in IOP was observed in contralateral eyes (Fig. 4A–C), in agreement with previous studies (Davis et al., 2016a). Topical instillation of CoQ10/TPGS or TPGS only micelles did not cause a significant change in IOP profile compared to untreated OHT, suggesting any other effects observed were independent of IOP (Fig. 4D).

Three weeks after surgical induction of OHT, animals had DARC imaging performed. The number of apoptotic RGCs was quantified from acquired retinal images by recording mean counts from two trained masked observers. A significantly lower number of apoptotic retinal cells was detected in OHT eyes treated with CoQ10/TPGS micelles compared to those treated with micelles containing only TPGS (one-way ANOVA with Tukey posthoc test, $p < 0.05$, Fig. 5). The number of apoptotic cells detected after treatment with CoQ10/TPGS micelles was similar to that detected in contralateral unoperated eyes.

RGC loss was evaluated by whole-retinal flat mounts labelled with Brn3a. CoQ10/TPGS but not TPGS treatment alone could protect rat retinal RGCs against IOP-induced apoptosis as indicated by the preservation in RGC density (Fig. 6A & B) and nearest neighbour distance (Fig. 6C & D) in the CoQ10/TPGS treated groups *versus* TPGS only or untreated (OHT only) controls.

4. Discussion

This study uses both *in vitro* and *in vivo* mitochondrial-mediated neurotoxicity models to successfully demonstrate the neuroprotective activity of CoQ10/TPGS compared to TPGS alone. Furthermore, twice-daily topical instillation of CoQ10/TPGS micelles was found to be significantly neuroprotective against RGC loss in a well-established rat model of OHT using *in vivo* and *ex-vivo* endpoints.

The findings also suggest that the antioxidant activity of TPGS alone

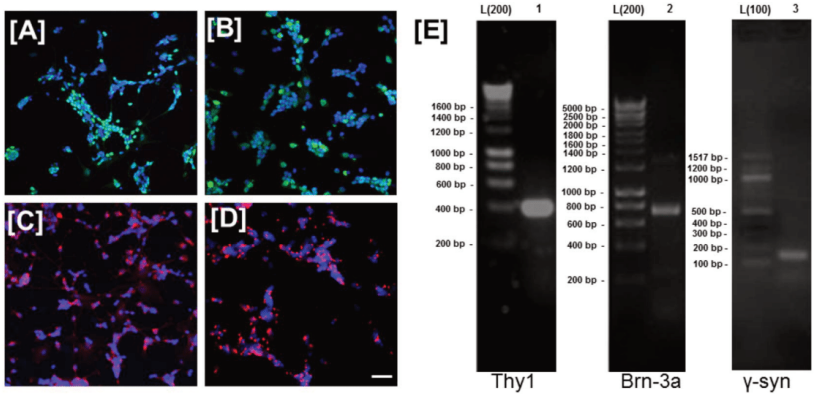
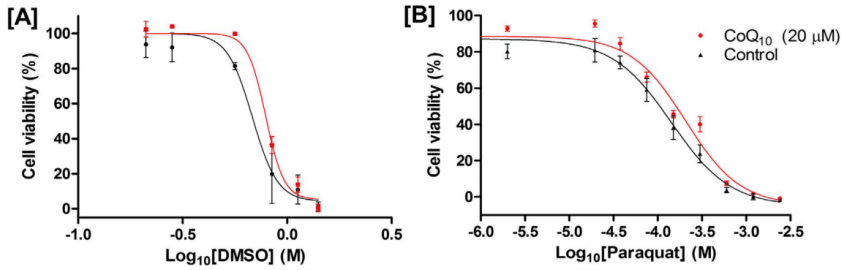


Fig. 2. Characterization of primary mixed murine retinal cultures enriched in RGCs. Immunostaining of primary murine cultures reveals a high concentration of cells labelled with RGC specific markers [A] Brn3a, [B] γ-synuclein, [C] RBPMS [D] Thy-1. Hoechst nuclear staining (blue) with immunostaining (FITC/TRITC). Scale bar = 20 μm, ×10 magnification. [E] Results were confirmed with reverse-transcriptase PCR using primers against (1) Thy-1, (2) Brn3a and (3) γ-synuclein. Band sizes were confirmed by comparison to appropriate molecular weight ladder; 200 bp (L200) or 100 bp (L100). No bands were detected in primary cell-free controls (data not shown) ruling out primer-dimers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Insult	Control IC_{50}	CoQ10/TPGS IC_{50}	TPGS IC_{50}	p value (Control vs CoQ10/TPGS)
DMSO (M)	0.668 ± 0.06	0.79 ± 0.05	0.70 ± 0.07	0.033
Paraquat (μM)	183 ± 67	312 ± 134	274 ± 128	0.0002

Fig. 3. CoQ10/TPGS micelles but not TPGS alone are neuroprotective against mitochondrial targeted cytotoxic insults in mixed murine retinal cultures containing RGCs. Pre-treatment of primary mixed murine retinal cell cultures with CoQ10/TPGS micelles (20 μM CoQ10 and 57 μM TPGS) but not equivalent concentrations of TPGS only significantly (two-way ANOVA, $p = 0.033$ and $p = 0.0002$ respectively) reduced the susceptibility of these cells to [A] DMSO and [B] paraquat-induced cytotoxicity.

was insufficient to protect an immortalised neuronal cell line from insults generating mitochondrial oxidative stress, such as DMSO and paraquat. This is in agreement with previous work which reported that co-administration of CoQ10 with the α-tocopherol derivative trolox enhances the neuroprotective activity of CoQ10 *in vitro* (Nakajima et al., 2008). The authors postulated the beneficial effect of vitamin E/CoQ10 co-therapy is a result of both agents having a synergistic antioxidant potential (Constantinescu et al., 1994). The reactivity of CoQ10 towards peroxyl radicals is reported to be much lower than that of α-tocopherol ($0.33 \times 10^4\ M^{-1}{\cdot}s^{-1}$ *versus* $3.3 \times 10^6\ M^{-1}{\cdot}s^{-1}$ respectively (Sohal, 2004)). However, the ability of CoQ10 to regenerate reduced α-tocopherol in the mitochondrial membrane (Sohal, 2004) and previous observations that supplementation with CoQ10 increases mitochondrial α-tocopherol concentration but not *vice-versa* (Lass and Sohal, 2000) could explain this effect. TPGS is not an antioxidant, first requiring decomposition by cellular esterases to liberate α-tocopherol, perhaps reducing the effective concentration of this antioxidant (Carini et al., 1990).

CoQ10 has been used in several treatment trials of retinal disorders. A randomized, double-blind, placebo-controlled clinical trial of 106 AMD (age-related macular degeneration) patients, reported improvements in visual function and retinal lesions after 12 months of oral CoQ10 therapy (20 mg/day) combined with mitochondrial targeting therapies Acetyl-L-Carnitine (200 mg/day) and n-3 Fatty acids (20 mg/day) (Feher et al., n.d.). More recently, the effects of topical CoQ10 therapy (2 drops/day) in combination with vitamin E TPGS (CoQun) was assessed in 22 open-angle glaucoma patients receiving β-blocker *versus* 21 patients receiving β-blocker monotherapy alone. This study reported a beneficial effect of CoQun therapy on electrophysiological functional tests including pattern electroretinography and visual cortical responses after 12 months Coqun therapy *versus* controls (Parisi et al., 2014).

The mechanism by which CoQ10 is thought to elicit neuroprotection is suggested to be a result of a combination of its well-documented antioxidant activity (Turunen et al., 2004), mechanical stabilisation of membrane structure reducing the risk of mitochondrial depolarisation (Sévin and Sauer, 2014) or *via* its Ca^{2+} buffering activity (Bogeski et al., 2011), important as an increase in intracellular Ca^{2+} is associated with apoptosis induction (Pinton et al., 2008). The ability of CoQ10 to inhibit glutamate excitotoxicity has been attributed to the reduction in expression of NR1 and NR2A subunits of *N*-methyl-D-aspartate receptor in the DBA/2J murine glaucoma model (Lee et al., 2014b). As both oxidative stress and glutamate excitotoxicity have been suggested to contribute to glaucoma pathogenesis, CoQ10 presents an intriguing glaucoma therapy (Davis et al., 2016c). Particularly as CoQ10 levels in the retina decline by approximately 40% with age, which may be associated with the onset of retinal disease (Qu et al., 2009).

In vitro, CoQ10 treatment was found to have a more pronounced effect on DMSO than paraquat IC_{50} values. A possible explanation for the observation is that while both DMSO and paraquat induce oxidative stress *via* affecting mitochondrial mediated respiration (Galvao et al., 2014; Yuan et al., 2014; Castello et al., 2007), we recently reported that DMSO can also induce an increase in cytoplasmic calcium resulting in BAX-mediated apoptosis induction. Coenzyme Q10 has recently been reported to bind and transport Ca^{2+} across membranes (Bogeski et al., 2011). The authors postulate that in addition to its anti-oxidant properties, coenzyme Q10 could therefore act as a cytosolic Ca^{2+}

Table 3
Mean IOP measurements and integral IOP (± SD) for each treatment group in this study.

Time post OHT induction (days)	OHT only	OHT (co-eye)	OHT + CoQ10/TPGS	OHT + CoQ10/TPGS (co-eye)	OHT + TPGS	OHT + TPGS (co-eye)
0	10.4	10.2	9.8	9.7	9.9	10.1
	(1.0)	(0.88)	(0.2)	(0.2)	(0.3)	(0.4)
1	20.6	10.4	17.8	11.6	19.0	11.1
	(3.8)	(1.7)	(2.7)	(0.9)	(2.8)	(1.5)
7	13	11.8	12.1	9.9	11.3	9.7
	(4.0)	(3.3)	(1.1)	(0.4)	(0.6)	(0.1)
21	11.2	11.1	11.6	10.2	10.0	9.5
	(2.0)	(1.2)	(1.5)	(0.8)	(0.6)	(0.3)
Integral IOP (mmHg/day)	286.0	237.3	270.2	216.2	255.1	206.9
	(42.4)	(43.0)	(27.3)	(7.7)	(14.5)	(5.4)

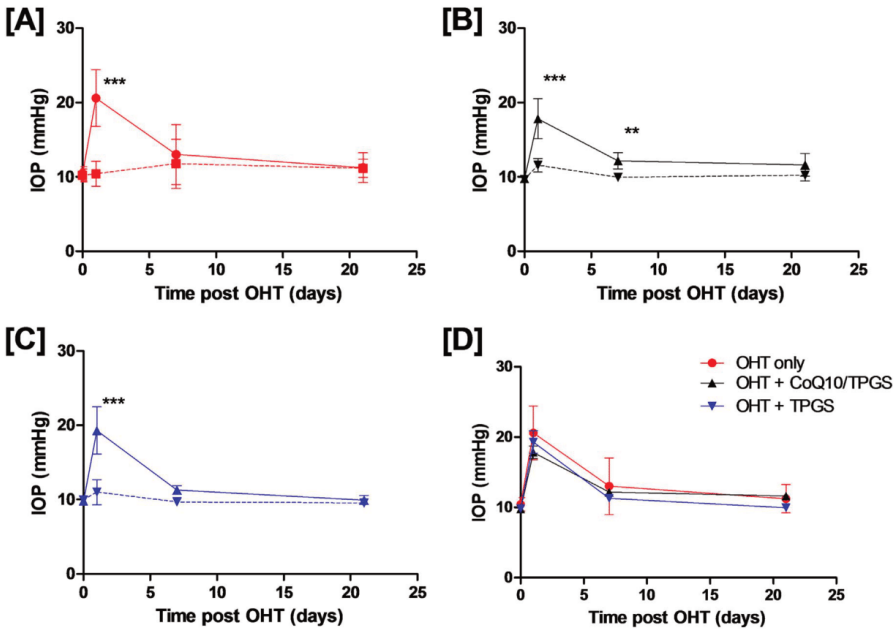


Fig. 4. CoQ10/TPGS or TPGS eye drop administration did not significantly affect IOP elevation induced by the OHT model. IOP profiles in DA rats after induction of OHT demonstrate a significant increase in IOP *versus* contralateral eyes [A–C] (Two-way repeated measures ANOVA with Bonferroni post-test, ***$p < 0.001$, **$p < 0.01$). Treatment of eyes with topical administration of CoQ10/TPGS [B] or TPGS only micelles [C] did not significantly alter the IOP profiles compared on OHT induction (two-way repeated measures ANOVA with Bonferroni post-test *versus* OHT model, $p > 0.05$) [D] suggesting any neuroprotective activity of treatments was a result of IOP independent effects. Results are mean ± SD.

buffer so protecting mitochondria from elevated cytosolic Ca^{2+} levels.

In support of a RGC neuroprotective mechanism, local ocular and systemic administration of CoQ10 (most commonly in conjunction with vitamin E derivatives) have been reported to offer retinal neuroprotective activity against models of retinal damage. Intravitreal and topical administration of CoQ10 has been reported to protect against retinal damage caused by IOP-induced ischemia or staurosporine by preventing glutamate-induced excitotoxicity and RCG apoptosis respectively (Nucci et al., 2007; Guo and Cordeiro, 2008). More recently, topical CoQ10 was found to elicit RGC neuroprotection over and above its antioxidant activity in a UV-induced rat model of retinal damage through inhibition of mitochondrial depolarization after topical instillation, (Papucci et al., 2003; Lulli et al., 2012) Nakajima et al. reported that systemic administration of CoQ10 (10 mg/kg) protected retinal cells against oxidative stress in an *in vivo* murine model of NMDA-induced retinal injury (Nakajima et al., 2008). Furthermore, in a transgenic DBA/2J murine glaucoma model, daily supplementation of the diet with 1% CoQ10 was found to promote RGC survival by 29% through decreasing Bax or increasing pBad protein expression and preserving mtDNA content and Tfam/OXPHOS complex IV protein expression in the glaucomatous retina (Lee et al., 2014b).

The protective effects of CoQ10 are not limited to neurons, with increasing reports that dietary supplement with CoQ10 therapy can also inhibit astroglial activation *via* mitochondrial-mediated effects, (Papucci et al., 2003; Lee et al., 2014b; Noh et al., 2013) which is increasingly recognised to play an important role in glaucoma pathology (Seitz et al., 2013). As a result, in addition to the aforementioned direct neuroprotective effects, CoQ10 may also elicit neuroprotective activity by acting on retinal glia. Administration of both DMSO (up to 5% v/v) and paraquat have previously been reported to promote astrocyte and glial toxicity *in vitro* (Yuan et al., 2014; Kim et al., 2008). Furthermore, subcutaneous administration of DMSO in P7 C57/BL/6By mice is reported to induce microglial activation in the brain (Saito et al., 2015) and administration of sub-toxic doses of paraquat in mice are reported to result in microglial activation prior to neurodegeneration (Purisai et al., 2007). In addition, a microglial inhibitory mechanism has recently been proposed in CoQ10 mediated protection against Aβ(1–42) induced cognitive dysfunction (Meneses et al., 2015) and pentylenetetrazol induced kindling epilepsy in mice (Bhardwaj and Kumar, 2016). In addition, with accumulating evidence for the involvement of amyloid beta in glaucoma pathology (Guo et al., 2007; Ito et al., 2012; Nizari et al., 2016) and growing recognition of mechanistic similarities between glaucoma and Alzheimer's disease (Gupta et al., 2016; Sivak, 2013), modulation of microglial activation by CoQ10 could contribute to the reported neuroprotective effects of this agent. Finally, reports of microglia activation in angiogenesis (Arnold and Betsholtz, 2013) and recent reports of microglial contribution to elevated basic fibroblast growth factor (bFGF) expression in the CNS after injury (Fujimaki et al., 2016) (perhaps *via* the ERK pathway (Lu et al., 2007; Ibrahim et al., 2011)), suggest a potential mechanism for the reported anti-angiogenic effects of CoQ10 (Choi et al., 2011; Jung et al., 2009; Sachdanandam, 2008) and its potential as a therapeutic for the treatment of age-related macular degeneration.

Beyond increasing the aqueous solubility and antioxidant potential of CoQ10, this study provides evidence to suggest that inhibition of P-gp activity may also play a role in the benefit of CoQ10/TPGS co-therapy. Inhibition of P-gp will reduce the efflux of extracellularly administered CoQ10, which is a recognised P-gp substrate (Hirano and Iseki, 2008). P-gp inhibition could, therefore, act to both increase the concentration of CoQ10 reaching intraocular tissues (*via* inhibition of P-gp in corneal epithelial cells, which contributes to the formidable corneal barrier to topically applied drugs (Dey et al., 2004)) and

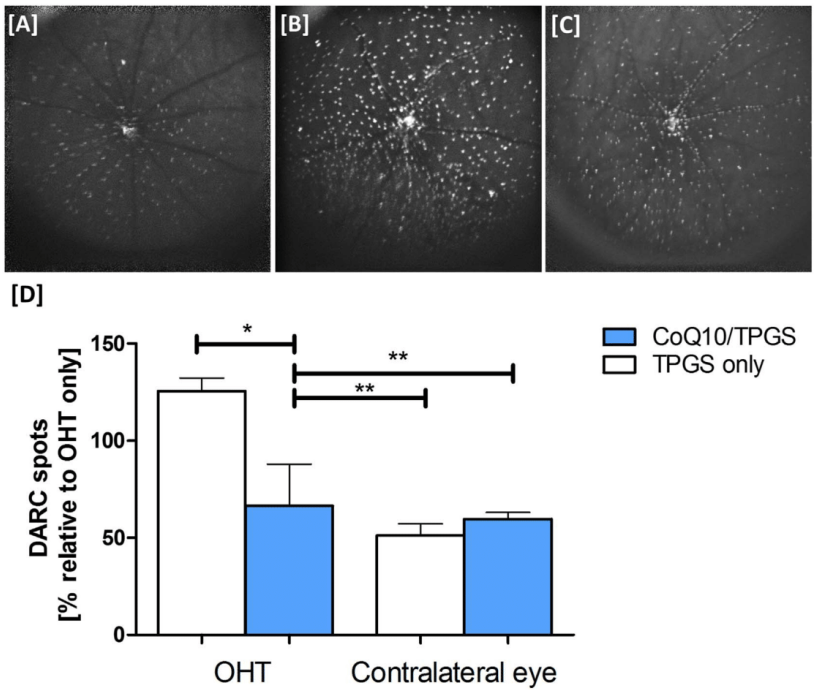


Fig. 5. *In vivo* detection of apoptotic retinal cells using DARC reveals CoQ10/TPGS micelles are significantly neuroprotective in the Morrison's OHT model. [A] Sample DARC image from CoQ10/TPGS treated DA rats exhibiting fewer apoptotic retinal cells (bright spots) than [B] eyes receiving TPGS only micelles or [C] OHT only eyes. [D] CoQ10/TPGS treatment was found to significantly reduce the DARC spot count when quantified by masked observers (one-way ANOVA with Tukey posthoc test, $p < 0.05$). Results are means ± SE.

impede the removal of CoQ10 from neuronal cells in the retina.

Although the P-gp inhibiting activity of TPGS is well-established (Dintaman and Silverman, 1999; Constantinides et al., 2006), the mechanism of action is poorly understood. TPGS is known not to interact directly with P-gp (Collnot et al., 2010), suggesting an indirect mechanism of action. There has been a recent growth in interest in the indirect modulation of membrane protein function *via* non-specific (Type II) lipid-protein interactions (Richens et al., 2015). The membrane dipole potential describes an electrical potential which arises from the restricted orientation of dipoles within membrane lipids and water molecules of the membrane solvation shell and has a magnitude of ~300 mV (O'Shea, 2003). The ability of α-tocopherol to modulate the membrane dipole potential of cholesterol containing membrane microdomains has recently been suggested as a possible mechanism of indirect P-gp activity modulation (Davis et al., 2015). In the present study, titration of TPGS in an immortalised neuronal cell line was found to induce a dose-dependent change in the membrane dipole potential, which fitted to a hyperbolic binding equation with a dissociation constant strikingly similar to the concentration of TPGS required to inhibit 50% of P-gp activity in the same cell line. Together, this data provides further evidence to support dipole potential modulation as a mechanism for α-tocopherol mediated P-gp inhibition.

Topical instillation of CoQ10/TPGS micelles but not TPGS micelles alone was found to significantly reduce the number of apoptotic retinal ganglion cells three weeks after induction of the OHT model without affecting IOP, suggesting an IOP independent neuroprotective effect of topical CoQ10 therapy. These results were confirmed with Brn3a whole mount histology which indicated almost complete protection of RGCs in the OHT retina upon treatment with CoQ10/TPGS micelles *versus* TPGS only or untreated groups. The results of this study provide evidence to support the use of the DARC technique to provide a quantitative assessment of retinal apoptosis and monitor the efficacy of therapeutic interventions *versus* appropriate controls. The impressive neuroprotective effect of CoQ10/TPGS may be a result of treatment commencing two days before OHT induction, suggesting this therapy may be most effective for patients at risk of IOP spikes such as following posterior capsulotomy or in pigment dispersion and Posner-Schlossman syndromes.

5. Conclusion

In conclusion, this study presents evidence that topically instilled CoQ10/TPGS micelles can deliver neuroprotective concentrations of these antioxidants to the retina *in vivo* using an established rodent model of ocular hypertension. These findings are in agreement with recent literature which suggests that this formulation can be used to deliver therapeutically relevant concentrations of CoQ10 to the posterior ocular tissues in humans after topical instillation (Fato et al., 2010) and suggest the potential utility of this neuroprotective therapies for the treatment of glaucoma.

Conflict of interest

MFC also holds patents pertaining the DARC technology. Visufarma holds patents regarding topical formulation of CoQ10.

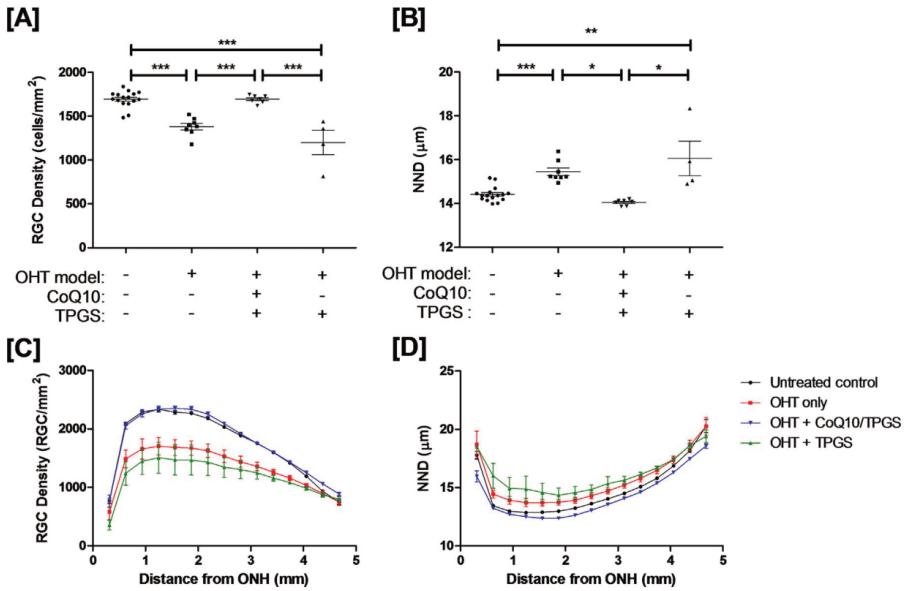


Fig. 6. Retinal ganglion cell survival after Morrison's OHT model in the DA rat is significantly enhanced by topical treatment with CoQ10/TPGS micelles. [A] OHT model induction led to a significant decline in RGC density (and increase in nearest neighbour distance) which was not recovered by treatment with TPGS micelles alone. Daily topical administration of CoQ10/TPGS micelles resulted in a significant preservation in RGC populations (one-way ANOVA with Tukey posthoc tests, $p < 0.001$). On dividing the retina into a series of 15 concentric non-overlapping rings (as described in (Davis et al., 2016a)), most pronounced RGC preservation occurs in the central retina [C, D].

Acknowledgements

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2.6. Neuroprotektive Wirkung von Curcumin in einem Glaukommodell

Davis BM, Pahlitzsch M, Guo L, Balendra S, Shah P, Ravindran N, Malaguarnera G, Sisa C, Shamsher E, Hamze H, Noor A, Sornsute A, Somavarapu S, Cordeiro MF. Topical Curcumin Nanocarriers are Neuroprotective in Eye Disease". Scientific Reports. 2018 Jul 23;8(1):11066. doi: 10.1038/s41598-018-29393-8.

Ein weiterer hochinteressanter Ansatz findet sich in der Wirkungsweise von Curcumin für eine neuroprotektive Therapie im Glaukom. Curcumin ist ein Polyphenol, das aus der Pflanzenfamilie Curcuma longa extrahiert wird.
Die klinische Verwendung von Curcumin wird vor allem durch die schlechte Löslichkeit und geringe Bioverfügbarkeit begrenzt.
In dieser Studie wird eine neuartige Nanopartikel-Formulierung eingesetzt, um die Bioverfügbarkeit des Curcumins zu erhöhen. In vitro Versuche zeigen, dass Curcumin-beladene Nanoträger signifikant vor Cobaltchlorid-induzierter Hypoxie und Glutamat-induzierter Toxizität schützen. Unter Verwendung etablierter in vivo Ratten-Glaukommodelle (OHT und partielle Sehnervdurchtrennung) kann durch eine Curcumin-Augentropfen Anwendung über drei Wochen zweimal täglich der Verlust der retinalen Ganglienzellen im Vergleich zu der Kontrollgruppe signifikant verringert werden. Die neue topische Curcumin-Formulierung ist somit eine wirksame neuroprotektive Therapie auf RGC in einem Glaukommodell.

Der nachfolgende Text entspricht dem Abstrakt des genannten Papers:

Originalsprachiges Abstrakt

„Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5dione) is a polyphenol extracted from turmeric that has long been advocated for the treatment of a variety of conditions including neurodegenerative and inflammatory disorders. Despite this promise, the clinical use of curcumin has been limited by the poor solubility and low bioavailability of this molecule. In this article, we describe a novel nanocarrier formulation comprising Pluronic-F127 stabilised D-α-Tocopherol polyethene glycol 1000 succinate nanoparticles, which were used to successfully solubilize high concentrations (4.3 mg/mL) of curcumin. Characterisation with x-ray diffraction and in vitro release assays localise curcumin to the nanocarrier interior, with each particle measuring <20 nm diameter. Curcumin-loaded nanocarriers (CN) were found to

significantly protect against cobalt chloride induced hypoxia and glutamate induced toxicity in vitro, with CN treatment significantly increasing R28 cell viability. Using established glaucoma-related in vivo models of ocular hypertension (OHT) and partial optic nerve transection (pONT), topical application of CN twice-daily for three weeks significantly reduced retinal ganglion cell loss compared to controls. Collectively, these results suggest that our novel topical CN formulation has potential as an effective neuroprotective therapy in glaucoma and other eye diseases with neuronal pathology."

SCIENTIFIC REPORTS

OPEN

Topical Curcumin Nanocarriers are Neuroprotective in Eye Disease

Benjamin M. Davis[1], Milena Pahlitzsch[1], Li Guo[1], Shiama Balendra[1], Parth Shah[1], Nivedita Ravindran[1], Giulia Malaguarnera[1], Claudia Sisa[1], Ehtesham Shamsher[1], Hisham Hamze[1], Abdinasir Noor[1], Acom Sornsute[2], Satyanarayana Somavarapu[2] & M. Francesca Cordeiro[1,3]

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Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5dione) is a polyphenol extracted from turmeric that has long been advocated for the treatment of a variety of conditions including neurodegenerative and inflammatory disorders. Despite this promise, the clinical use of curcumin has been limited by the poor solubility and low bioavailability of this molecule. In this article, we describe a novel nanocarrier formulation comprising Pluronic-F127 stabilised D-α-Tocopherol polyethene glycol 1000 succinate nanoparticles, which were used to successfully solubilize high concentrations (4.3 mg/mL) of curcumin. Characterisation with x-ray diffraction and *in vitro* release assays localise curcumin to the nanocarrier interior, with each particle measuring <20 nm diameter. Curcumin-loaded nanocarriers (CN) were found to significantly protect against cobalt chloride induced hypoxia and glutamate induced toxicity *in vitro*, with CN treatment significantly increasing R28 cell viability. Using established glaucoma-related *in vivo* models of ocular hypertension (OHT) and partial optic nerve transection (pONT), topical application of CN twice-daily for three weeks significantly reduced retinal ganglion cell loss compared to controls. Collectively, these results suggest that our novel topical CN formulation has potential as an effective neuroprotective therapy in glaucoma and other eye diseases with neuronal pathology.

Glaucoma describes a distinctive group of progressive optic neuropathies affecting over 60 million people worldwide and responsible for 8.4 million cases of irreversible blindness[1]. Although several mechanisms have been proposed, glaucoma principally involves the loss of retinal ganglion cells (RGCs)[2]. Elevated intraocular pressure (IOP) today presents the only clinically modifiable risk factor for glaucoma progression[3], however, many patients continue to lose visual field despite well controlled IOP[4]. Due to the limited effectiveness and indirect nature of IOP modulating therapy, the development of novel therapeutic approaches for the treatment of glaucoma independent of IOP modulation is now sought[5].

RGC apoptosis has been identified as an early event in glaucomatous degeneration and the inhibition of this process has been advocated as a therapeutic strategy[5,6]. For example, in addition to the well-established IOP modulatory effects of brimonidine[7], this third generation α_2 adrenergic agonist is also reported to possess an additional neuroprotective activity in rodent models of glaucoma[8]. Although the mechanism of action remains to be fully described, there is emerging clinical evidence to suggest that topical brimonidine therapy exhibits an RGC preserving activity over and above IOP modulating effects in patients with primary open-angle glaucoma[9].

Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5dione) is a polyphenol extracted from turmeric (*Curcuma longa*)[10] reported to modulate a range of biochemical processes implicated in neurodegenerative disorders[11]. For example, curcumin has been found to attenuate pathways implicated in the pathogenesis of the most common ophthalmic disorders[12–19], including: mitochondrial-mediated oxidative stress[20], inflammatory responses via PPAR-γ agonist activity[21], down-regulation of COX-2 and iNOS[22], downregulation of JAK2-STAT3 mediated astrogliosis[23], β-amyloid aggregation[24], and anti-angiogenic activity via modulation of the VEGF/VEGFR/K-ras pathway[25]. Supplementing rodent diets with 0.01% to 0.25% curcumin has previously been reported to protect RGCs and microvasculature against ischemia/reperfusion injury via inhibition of Nf-κB, STAT3 and MCP-1 overexpression[26]. More recently, intragastric administration of curcumin (10 mg/kg/day) for 6 weeks in a rodent model of ocular hypertension was reported to result in a significant reduction in retinal

[1]UCL Institute of Ophthalmology, London, EC1V 9EL, United Kingdom. [2]UCL School of Pharmacy, London, WC1N 1AX, United Kingdom. [3]Western Eye Hospital, ICORG, Imperial College London, London, NW1 5QH, United Kingdom. Correspondence and requests for materials should be addressed to M.F.C. (email: m.cordeiro@ucl.ac.uk)

microglial death[27]. This equates to a typical human dose of 800 mg/day which has previously been associated with adverse effects such as nausea and diarrhoea in addition to an increase in serum alkaline phosphatase and lactate dehydrogenase levels[28]. Despite the therapeutic potential of curcumin in ophthalmology, however, several key challenges have limited the clinical applicability of this agent including its poor water solubility (~11 ng/mL)[29] and low bioavailability[10,30]. Single oral administration of between 2 g and 12 g of curcumin in humans yields peak serum concentrations of less than 50 ng/mL[31,32] and repeated administration of gram doses (0.4 g to 12 g) yields variable peak plasma concentrations typically less than 1 μg/mL, with no accumulation after repeated daily administration for up to 3 months[28,33,34]. The number of capsules each patient was required to take (up to 24 × 500 mg capsules per day) and mild gastrointestinal side-effects precluded higher oral dosing of curcumin in this form.

For treatment of ophthalmic disorders, topical administration is the preferred delivery route as it permits self-administration and localises dosing to ocular tissues, minimising the risk of side effects associated with systemic absorption. Local dosing also offers a method to overcome the extremely low systemic bioavailability, rapid metabolic degradation and clearance of this agent[35]. For instance, in mice only trace amounts (0.41 μg/g) of curcumin are reported to reach the CNS after systemic administration[36]. As typically less than 3% of topically applied small drugs are reported to reach posterior ocular tissues[37], the extremely limited water solubility of curcumin has been a challenge for the topical administration of this drug. Methods to enhance the solubility of curcumin include; prior solubilization in an alkaline buffer (typically 0.5 M sodium hydroxide), dissolution in solvents such as DMSO or incorporation into nanocarriers[38–44]. Solvents such as DMSO are commonly used to dissolve poorly soluble drug candidates for preclinical investigation, however, the toxicity of this agent, even at low concentrations, is increasingly recognised[45]. Furthermore, dissolution of curcumin in DMSO or alkaline are unsuitable for *in vitro* and *in vivo* applications without further dilution into physiological buffers, and subsequent dilution of these curcumin solutions into aqueous buffers at physiological pH, are frequently unstable and result in rapid precipitation[46].

To overcome these limitations, nanotechnology approaches can be used to provide hydrophobic environments for poorly soluble drug molecules which persist in a stable aqueous suspension. Further advantages of this approach include nanoparticle-mediated protection of encapsulated drug cargo from hydrolytic or enzymatic degradation and enhanced transport across biological barriers[47]. D-α-tocopherol polyethene glycol 1000 succinate (TPGS) is a non-ionic surfactant that forms stable micelles at concentrations of greater than 0.02% w/w[48]. TPGS is considered a safe pharmaceutic adjuvant by the FDA, which coupled with the observation that this agent can inhibit P-glycoprotein activity[49] has led to the widespread use of this agent in drug delivery systems[50]. In this study, TPGS was combine with Pluronic F127, a difunctional block copolymer surfactant consisting of a central hydrophobic polyoxypropylene group flanked by hydrophilic polyoxyethylene groups. Pluronic F127 has previously been used to sterically stabilise nanocarriers against aggregation[51]. This, combined with the thermo-responsive properties and high biocompatibility of this polymer, has led to it being widely used for ophthalmic drug delivery applications[52,53].

The aim of the present study was to develop a curcumin nanocarrier comprising TPGS and Pluronic F127 suitable for use as a topical formulation in the treatment of eye diseases. We describe the development of a novel CN formulation of curcumin solubilizing up to 4.5 mg/mL of curcumin with an encapsulation efficiency exceeding 95%, average particle size <20 nm and good stability for over two months when stored at 25 °C in liquid or lyophilized forms. The neuroprotective potential of this formulation is then assessed in the immortalised R28 retinal precursor cell line[54] subject to insults that have previously been suggested to model aspects of the retinal environment in glaucoma. These include; cobalt chloride (hypoxia mimetic)[55] and glutamate induced toxicity[56]. Finally, this formulation was shown to be effective as an eye-drop in reducing RGC loss in two well-established rodent models of optic nerve disease, ocular hypertension (OHT) and partial optic nerve transection (pONT) models[57].

Results and Discussion

Spectroscopic methods can be used to assess curcumin encapsulation efficiency and oxidation state. On dilution in dimethyl sulfoxide (DMSO) curcumin had an absorbance peak at 435 nm (Fig. 1C) and molar extinction coefficient (Fig. 1D,E) of 58547 $L.mol^{-1}.cm^{-1}$, comparable to previously reported values[58]. The absorbance of curcumin diluted in DMSO at 435 nm obeyed Beer-Lambert's law up to 42 μM and TPGS/Pluronic F127 nanocarriers in the absence of curcumin had no measurable absorbance at this wavelength. Spectroscopic assessment was used to determine the encapsulation efficiency (EE%) of curcumin-containing formulations after separation of unencapsulated material by 0.22 μm filtration. Spectroscopic measurements of EE% were confirmed using an established HPLC technique (Fig. 1F) with both techniques showed good agreement (4.31 ± 0.18 mg/mL versus 4.32 ± 0.33 mg/mL respectively).

Spectroscopic determination of curcumin concentration in nanocarriers can also be used to give indication of the extent of curcumin degradation. Curcumin undergoes keto-enol tautomerization (Fig. 1A), existing in the more stable keto form under acidic or neutral conditions and the more water soluble enol-form under alkaline conditions. In common with other molecules that undergo keto-enol tautomerization[59], the enol form of curcumin is more prone to hydrolytic degradation[60]. Acceleration of curcumin degradation processes by dissolution in an alkaline buffer[58], gave rise to a dramatically reduced curcumin molar extinction coefficient at 435 nm compared to formulated curcumin (Fig. 1C,D, 2133 $L.mol^{-1}.cm^{-1}$ after 72 h incubation in the presence of 1 M sodium hydroxide solution). Furthermore, incubation of CNs in alkaline conditions induced a dramatic colour change from orange to brown (Fig. 1E). Spectroscopic assessment of curcumin concentration after dissolution in sodium hydroxide indicates that the curcumin molar extinction coefficient rapidly diminished, suggesting that this technique can not only be used to assess curcumin entrapment efficiency but also be used to monitor the extent of degradation of curcumin containing formulations.

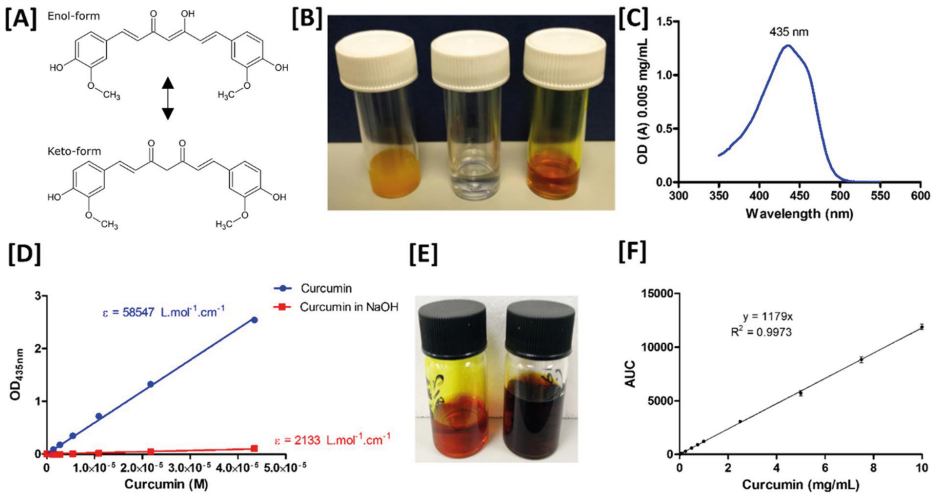

Figure 1. Spectroscopic determination of curcumin content of nanocarrier formulations. (**A**) The keto- and enol- forms of curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione). (**B**) Suspensions of 4.5 mg/mL of curcumin in *(left)* PBS, *(centre)* PBS after 0.2 µm filtration to remove insoluble material and *(right)* in TPGS/Pluronic F127 nanoparticles after 0.2 µm filtration to remove insoluble material. (**C**) On dissolution in DMSO, 22 µM curcumin possesses an absorption peak of 435 nm. (**D**) Determination of the molar extinction coefficient of curcumin in a DMSO solvent (58,547 $L.mol^{-1}.cm^{-1}$) and demonstration that this accelerated oxidative degradation of curcumin at low pH[92] results in a reduction in this molar extinction coefficient (2133 $L.mol^{-1}.cm^{-1}$ after 72 h incubation in 1 M sodium hydroxide solution) suggesting that spectroscopic assessment can be used to monitor both the encapsulation of curcumin and its degradation. (**E**) Dissolution of 5 mg/mL curcumin in 1 M sodium hydroxide solution *(right)* resulting in a rapid colour change compared to curcumin loaded nanocarriers *(left)*. (**F**) Standard curve of known concentration of curcumin measured by HPLC.

TPGS/Pluronic F127 nanocarriers enhance curcumin solubility and stability. Initially, curcumin loaded nanocarriers were prepared by incorporation it into TPGS nanocarriers. TPGS was chosen due to the low critical micelle concentration of this excipient (0.02% w/w)[48], the endogenous nature and antioxidant properties of the α-tocopherol component[61] and P-glycoprotein antagonism[49], which enhances the barrier crossing ability of formulations containing this agent[62]. TPGS is present in existing ophthalmic formulations[62] and both curcumin and TPGS can be readily solubilized in ethanol, a solvent which is present at concentrations of 0.8% in commercially available eye drop formulations (i.e. Optrex ActiMist 2in1 Eye Spray for Dry Irritated Eyes) so reducing risks associated with residual solvents from the manufacturing process. Furthermore, as the use of TPGS to enhance the bioavailability of orally administered drugs is well documented[60]. This, in combination with recent interest in the use of Pluronic F127 food-research applications[63] may suggest that the novel curcumin formulation described herein may also be suitable for oral administration.

Formulation of curcumin with TPGS micelles was found to produce nanocarriers with 16 nm diameter as determined by dynamic light scattering (data not shown). Unfortunately, these formulations rapidly aggregated at 25 °C, resulting in the formation of sediment within hours of resuspension which may be indicative of Ostwald ripening processes[64]. Stabilisation of curcumin loaded TPGS nanocarriers was achieved by the addition of the polymeric stabiliser Pluronic F127 (a triblock copolymer of polyoxyethylene and polyoxypropylene), which has previously been used to sterically stabilise nanocarriers against aggregation[51].

Curcumin-loaded nanocarriers (CN) were prepared according to the methods described, with encapsulation efficiency and average particle size determined (Fig. 2). On resuspension in PBS (pH 7.4) or HEPES trehalose buffer (pH 7.4), nanocarriers were found to encapsulate 96.0% ± 2.0% (4.32 mg/mL) and 94.2% ± 4.1% (4.31 mg/mL) of curcumin respectively. Transmission electron microscopy revealed that nanocarriers were typically 20 nm in diameter and of uniform size (Fig. 2A). These results were confirmed by dynamic light scattering (Fig. 2B,C) which identified a homogeneous particle dispersion with a z-average diameter between 16 and 20 nm suggestive of a micellar formulation.

The encapsulation efficiency and particle size of CN formulations were assessed over time after storage at 25 °C while protecting from light. The CN formulation was found to exhibit excellent stability for 9 weeks at 25 °C, with no reduction in formulation EE% (Fig. 2E), significant change in particle diameter (Fig. 2F) or dispersity (Fig. 2G) over this time. This stability study was repeated using lyophilised CNs prepared in the same buffer before storing at 25 °C while protecting from light. The residual water content calculated at 120 °C was 1.085 ± 0.050%, indicating lyophilized formulations were properly prepared. Formulations were resuspended prior to recording

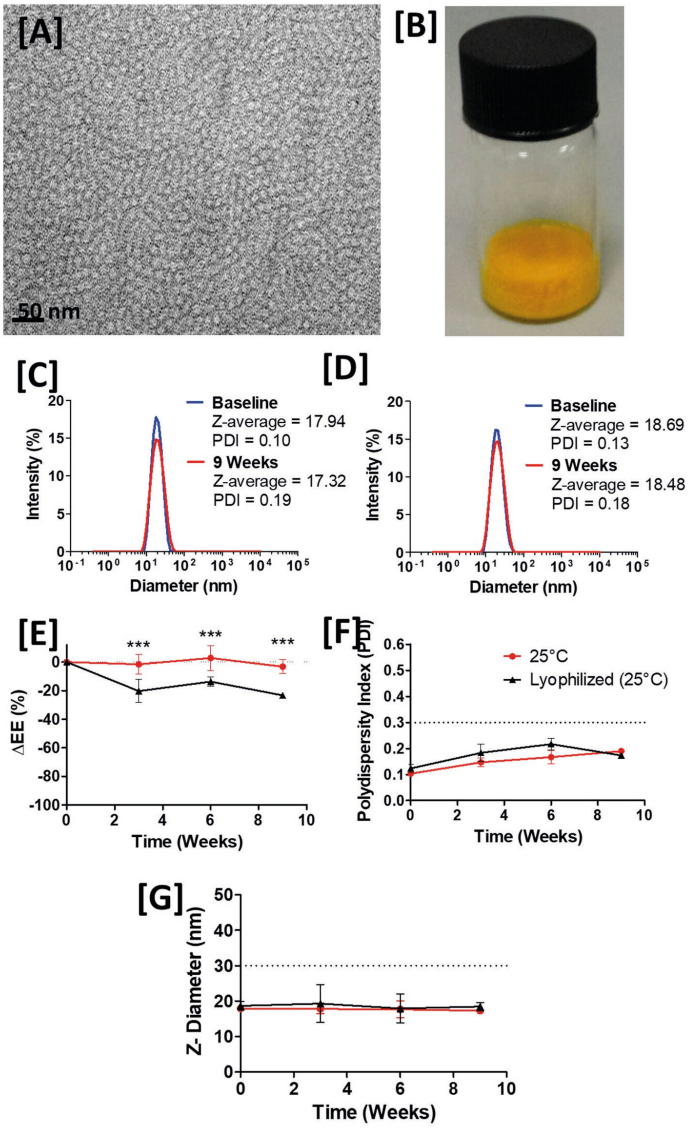

Figure 2. Characterization of curcumin loaded nanoparticles and stability assessment over time. (**A**) Transmission Electron Micrograph of curcumin loaded nanoparticles (CNs) negatively stained with 1% Uranyl acetate. Scale bar = 50 nm. Dynamic light scattering revealed a homogeneous particle population which did not significantly change on storage at (**B**) 25 °C or (**C**) on lyophilization and storage at 25 °C and resuspension after 9 weeks. (**D**) Photograph of 1 mL lyophilized CN in 10 mM HEPES, 50 mg/mL trehalose buffer showing good cake structure. Stability studies illustrating the change in encapsulation efficiency over time when CN were stored at (**E**) 25 °C (solution) versus lyophilized and rehydrated. The average particle size (**F**) and dispersity index (**G**) was recorded in each case. Mean ± 95% CI.

dispersion properties (Fig. 2E,G) which were found to remain constant and similar to those reported for liquid formulations (Table 1). EE% was found to decline by an average of 20% versus baseline at each time point assessed, suggesting this may be a result of the lyophilization or rehydration process.

Mean (SD)	Baseline		Three Weeks		Six Weeks		Nine Weeks	
Storage	25 °C	Lyophilized	25 °C	Lyophilized	25 °C	Lyophilized	25 °C	Lyophilized
EE (%)	94.2 (4.1)	101.6 (6.7)	92.6 (1.7)	81.2 (9.7)	97.1 (1.1)	87.8 (1.6)	91.0 (2.2)	78.2 (7.4)
Z-Diameter (nm)	17.9 (0.4)	18.7 (0.5)	17.8 (0.5)	19.3 (2.1)	17.7 (1.0)	17.9 (1.6)	17.3 (0.1)	18.5 (0.5)
PDI	0.002 (0.004)	0.128 (0.029)	0.146 (0.027)	0.183 (0.060)	0.168 (0.046)	0.218 (0.038)	0.188 (0.001)	0.177 (0.0011)

Table 1. Characteristics of curcumin loaded nanocarriers and stability over time (n = 3).

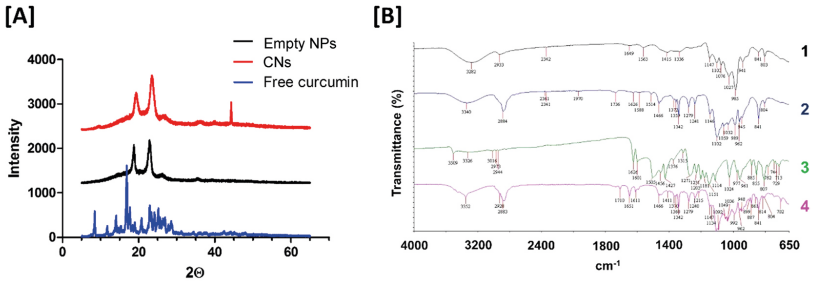

Figure 3. X-ray diffraction and FT-IR characterization of curcumin loaded nanocarrier formulations. (**A**) X-ray diffraction pattern of naïve curcumin (blue), empty nanocarriers (black) and curcumin nanocarriers (red). (**B**) FTIR analysis of (1) trehalose, (2) curcumin loaded nanoparticles, (3) free curcumin and (4) empty nanoparticles.

Several groups have previously attempted to prepare curcumin loaded nanoparticle formulations, including; PLGA-nanocarriers[38,39], solid lipid nanocarriers[40,41], liposomes[42,43] and exosomes[44]. Existing nanoparticulate formulations of curcumin possess limited stability (not assessed beyond 72 h in any study cited), only moderate curcumin loading has been achieved (<0.77 mg/mL)[38] and most protocols would be difficult to translate to the clinic owing to complex, multi-step manufacture protocols requiring organic solvents. The TPGS/Pluronic F127 curcumin formulation described here compares favourably with those in the existing literature.

XRD and FT-IR spectra were acquired to ascertain the nature of curcumin once incorporated into nanocarriers (Fig. 3). The X-ray diffraction patterns of free curcumin exhibited characteristic peaks between 5° and 30°, indicative of a highly crystalline structure[65]. This character was lost on inclusion of curcumin in a nanocarrier formulation, indicating that curcumin has successfully been incorporated into the amorphous nanocarrier structure and is not associated with the particle surface[66]. FT-IR spectra reveal characteristic peaks of free curcumin at 3509 cm^{-1}, 1626 cm^{-1}, 1601 cm^{-1}, 1505 cm^{-1}, 1271 cm^{-1}, 1024 cm^{-1}, 948 cm^{-1} and 713 cm^{-1} which closely match previously reported values[67]. On incorporation into nanocarriers, the characteristic curcumin peak at 3509 cm^{-1} (indicative of the free hydroxyl group) merged with the broad OH peak of the TPGS/Pluronic F127 carrier at 3352 cm^{-1}, which may suggest complex formation[68]. Furthermore, characteristics shifts in the aromatic C=C peak (1601 cm^{-1} to 1588 cm^{-1}) and the C=O stretching, δ(CCC) and δ(CCO) in plane bending from 1505 cm^{-1} to 1514 cm^{-1} have previously been interpreted as evidence for the successful incorporation of curcumin into a complex[68].

Formulation of curcumin into nanocarriers substantially reduced the rate of drug release compared to free drug ($t_{1/2}$ = 22.6 h versus 0.15 h respectively, Fig. 4) at 37 °C, attributed to the slow rate of release of curcumin from nanocarriers. Less than 10% of the drug was liberated after 5 h of incubation, suggesting that there was little burst release from the CN formulation. This observation supports FT-IR and XRD observations that curcumin is not merely associated with the nanocarrier surface but is localised within the hydrophobic interior in an amorphous or disordered crystalline phase, in agreement with previous work[41]. Together, these results suggest that the curcumin-loaded nanocarrier formulation described in this study have sustained release capability.

Curcumin-loaded nanocarriers protect a retinal cell line against glutamate and hypoxia-induced injury. Glutamate excitotoxicity represents a potential mechanism leading to RGC loss in glaucoma[69,70]. Using an AlamarBlue cell viability assay, co-incubation of immortalised R28 cells with both CNs and empty nanoparticles was found to be significantly protective (one-way ANOVA with Tukey post-test, $p < 0.001$) against glutamate induced toxicity (Fig. 5A and B, IC_{50} 28.3 ± 3.4 mM versus 5.9 ± 1.2 mM for EM and insult only treated groups respectively, on-way ANOVA with Tukey post-test $p < 0.001$) with no additive effect observed on addition of curcumin to the nanoparticles (24.5 ± 1.2 mM, CN containing 20 μM curcumin). This observation is in agreement with previous studies that suggest α-tocopherol (here present in the form of TPGS) is protective against glutamate induced toxicity and this has been suggested to be a result of the anti-oxidant function of this molecule[71,72]. As TPGS was not also protective against cobalt chloride induced insult, this suggests curcumin and TPGS may have additive therapeutic effects.

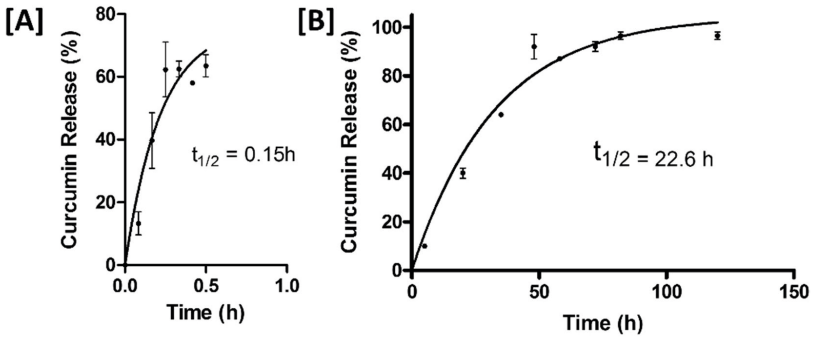

Figure 4. *In vitro* release of curcumin. *In vitro* release of 4.5 mg/mL curcumin from (**A**) 95% ethanol solution or (**B**) curcumin-loaded nanocarriers in PBS at 37 °C (mean ± SE, n = 3). Owing to the poor solubility of curcumin in aqueous buffers, the release of curcumin from ethanoic solutions was limited by the formation of a visible precipitate from the 0.5 h time point. No such aggregation was observed in experiments using nanocarrier curcumin.

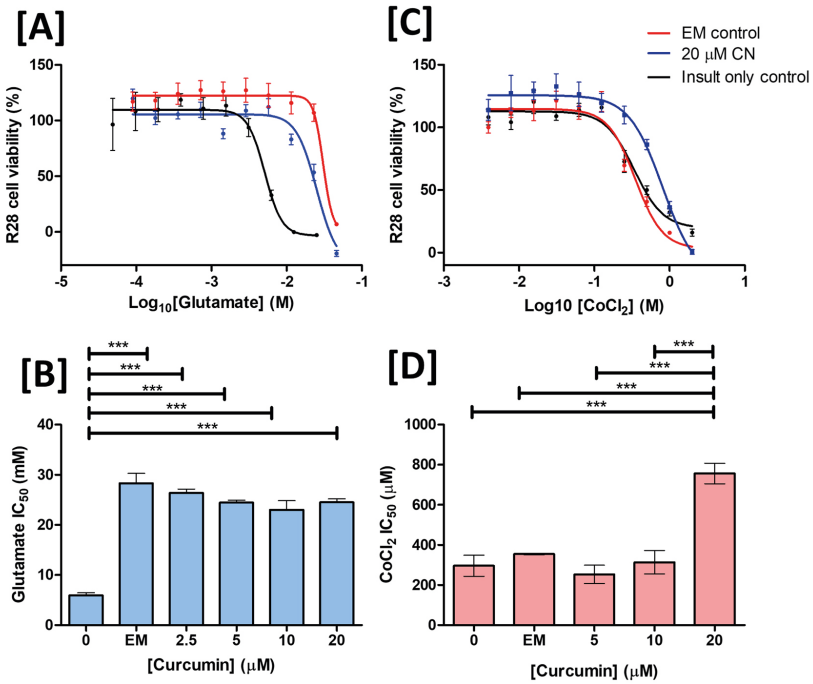

Figure 5. Curcumin nanoparticle treatment is neuroprotective against the hypoxia mimetic cobalt chloride in immortalized retinal cells. Using an alamarBlue cell viability assay, Co-incubation of R28 cells with varying concentration of CNs significantly protected cells against (**A**,**B**) glutamate or (**C**,**D**) cobalt chloride induced insult (one-way ANOVA with Tukey post-test, ***$p < 0.001$). Empty nanoparticles containing TPGS were found to be neuroprotective against glutamate induced toxicity (**B**) but not cobalt chloride (**D**) induced toxicity.

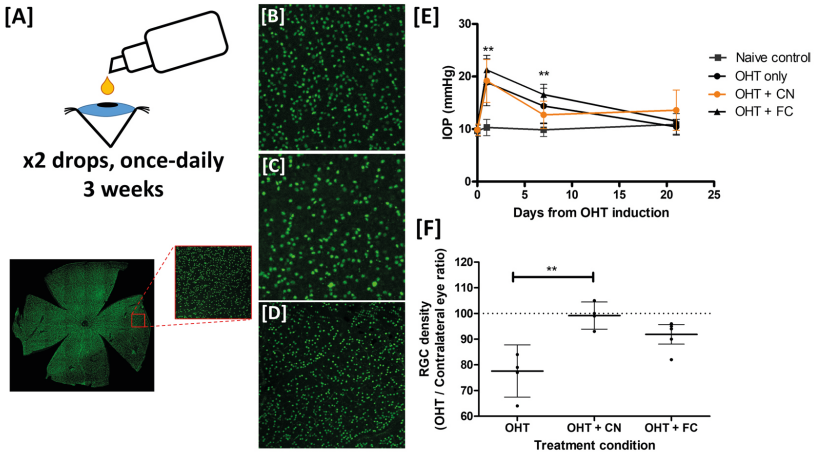

Figure 6. Topical curcumin nanoparticles protect RGC soma *in vivo* against OHT induced cell loss. (**A**) Schematic of *in-vivo* experimental design. OHT rats were randomised to no treatment or once-daily curcumin nanoparticles (CN) or free Curcumin (FC) eye-drops, beginning two days prior to elevated IOP induction. Three-weeks after surgery, animals were sacrificed and retinas flat mounted before labelling with Brn3a. RGC populations were counted as previously described[57,94]. Representative retinal images of comparable Brn3a labelled areas of superior retina are shown in (**B**) Naïve control, (**C**) OHT untreated and (**D**) OHT + CN animals. (**E**) All OHT animals had significantly raised IOP (mean ± SE) versus baseline until 21 days after surgery (Student T-test versus contralateral eyes $^{**}p < 0.01$). There was no significant difference in IOP between OHT treatment groups at any time point suggesting any neuroprotective activity observed was IOP independent. (**F**) Elevated IOP in OHT only eyes was associated with a significant reduction in RGC density (~23%) in agreement with previous studies[57]; CN but not FC treatment, significantly reduced RGC loss (Kruskal-Wallis test with Dunns post test, $^{**}p < 0.01$).

Upregulation of hypoxia-related factors such as Hypoxia Inducible Factor 1α (HIF-1α) has been suggested to implicate hypoxia in glaucoma pathology[73,74]. Cobalt chloride ($CoCl_2$) is a hypoxia mimetic and inducer of HIF-1α[75] used as an *in vitro* glaucoma model[76]. The IC_{50} of R28 cells exposed to $CoCl_2$ for 24 h (Fig. 5C,D) was found to be significantly increased on concurrent incubation with 20 μM curcumin in the form of CN (296 ± 53 μM vs 757 ± 51 μM respectively, one-way ANOVA with Tukey post-test, $p < 0.001$). Treatment with an equivalent concentration of the nanoparticle in the absence of curcumin had no significant effect (296 ± 53 μM versus 354 ± 8 μM, one-way ANOVA with Tukey post-test, $p > 0.05$) suggesting that the protective effects observed were as a result of curcumin. Concentrations of curcumin <20 μM were not found to be neuroprotective in this model. Curcumin has previously been reported to inhibit HIF-1α in hepatocellular carcinoma cells[77] and was more recently reported to supresses HIF-1α synthesis in pituitary adenomas[78]. HIF-1α inhibitors have previously been proposed as potential glaucoma treatment worthy of further investigation[79].

Topically administered curcumin nanocarrier therapy protects RGCs in rodent models of ocular hypertension and optic nerve injury. Having established the neuroprotective activity of CNs *in vitro* in relation to vehicle only treatments, we next assessed the neuroprotective effects of this formulation on RGC health using an established *in vivo* rodent model of RGC loss. We anticipate that topically applied curcumin loaded nanoparticles will reach the retina via a combination of topical and systemic absorption routes. In support of this hypothesis, Sigurdsson *et al.* reported that their formulation of dexamethasone, which is a similar molecular weight to curcumin (392 versus 368 Da respectively), entered the retina 60% via topical penetration and 40% by systemic absorption route[80]. We anticipate that the well-documented P-gp inhibition activity of tocopherols[49,81] and curcumin[82], in conjunction with enhanced corneal penetration activity previously reported for PEGylated-micelle formulations[83] will enhance curcumin delivery to the retina by the topical absorption route.

Optimum time points post model induction (maximal RGC loss in shortest time after induction) were chosen based on our previous work characterising the natural history of the OHT and pONT models where multiple time points were assessed after model induction[57]. We recently reported that administration of TPGS containing micelles did not themselves have a neuroprotective effect *in vivo*[81], which in conjunction with our *in vitro* observations, suggest that any neuroprotective efficacy observed was a result of curcumin treatment. Rats received topical CNs according to the dosing regimen illustrated in Fig. 6A. Briefly, two days prior to OHT model induction, rodents began receiving two drops (35 μL each) of CNs dosed five minutes apart per day for three weeks from the date of model induction. Topical administration of CNs was found to be well-tolerated by rats with no signs of ocular irritation or inflammation reported in naive eyes monitored by a qualified ophthalmologist. The

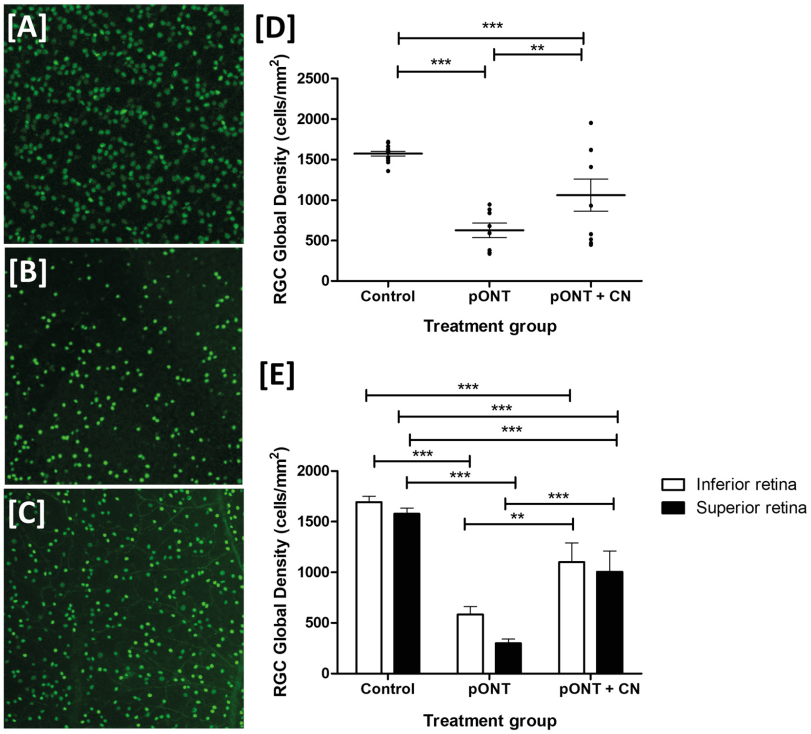

Figure 7. Topical curcumin nanoparticles protect RGC soma against optic nerve injury. Representative Brn3a labelled superior retinal sections taken from similar areas in (**A**) Naïve retina (**B**) pONT retina and (**C**) pONT model retina after daily topical CN treatment for 21 days. (**D**) Whole retinal RGC density measurements indicate that while pONT caused a significant reduction in RGC density, this was reduced by daily administration of CN (one-way ANOVA with Tukey post hoc test, $^{**}p < 0.01$ and $^{***}p < 0.0001$). (**E**) Further segmentation of the each retina into superior and inferior quadrants using the method described previously[57] demonstrates that topical CN prevent some RGC density loss in both the superior and inferior retina (two-way ANOVA with Tukey post hoc tests $^{***}p < 0.001$).

IOP profile of rodent's after IOP elevation by injection of hypertonic saline into two episcleral veins (Fig. 6B) indicates that IOP remained significantly elevated for at least 7 days after model induction versus naive eyes. No significant difference in IOP profile between CN and OHT only groups was observed, suggesting that any neuroprotective effect of curcumin was due to IOP independent processes. RGC health was assessed histologically from whole-retinal mounts using brn3a assessment (Fig. 6C). This approach was chosen as Brn3a is a nuclear restricted and RGC specific transcription factor that exclusively label 97% of the RGC population (excluding photosensitive RGCs)[84]. We have also recently developed an algorithm to accurately and automatically quantify whole RGC populations in rodent models of retinopathy enabling the reliable assessment of RGC health[57]. Using this approach, OHT induction was found to result in a significant reduction in global RGC density of ~23% compared to contralateral eyes, which is comparable to previous studies using this model[57]. CN administration significantly improved the RGC density ratio between OHT eye vs contralateral untreated eyes (Kruskal-Wallis test with Dunns post test, $^{**}p < 0.01$), whereas administration of un-encapsulated curcumin (FC - free curcumin) solubilised in PBS did not have this effect (Fig. 6D–F).

To further investigate the neuroprotective potential of topically applied CNs, whole-retinal brn3a labelled RGC population assessments were made in the pONT model (Fig. 7A). In this model, twice-daily topical administration of CNs was found be significantly protect RGCs (one-way ANOVA, $^{***}p < 0.001$). On subdivision of whole retinal mounts into superior and inferior quadrants (Fig. 7B), treatment with CNs was observed to result in preservation of RGC populations in both the superior and inferior quadrants, but this effect was more pronounced in the superior retina (two-way ANOVA, $^{***}p < 0.001$), which may imply the protective effects of curcumin therapy exert through anti-apoptotic as well as anti-oxidant mechanisms. Representative regions from the superior quadrant of Brn3a labelled retinal whole-mounts (Fig. 7C–E) illustrate RGC populations

were diminished in retina subject pONT (Fig. 7D) versus naive controls (Fig. 7C). Treatment with CNs for three weeks was found to protect RGC soma from pONT induced injury (Fig. 7E). As preservation of RGC soma was observed in both the superior and inferior retinal quadrants, this suggests that curcumin may elicit neuroprotective activity via multiple pathways involving both primary and secondary neurodegeneration processes.

The possibility of TPGS mediated neuroprotection via inhibition of glutamate excitotoxicity is intriguing and may contribute to the neuroprotective effect of our formulation *in vivo*. In support of this hypothesis and our present *in vitro* findings, Nucci *et al.*[85] previously reported that intraocular administration of a total of 10 µL of 0.5% (w/v) TPGS (equivalent to a total dose of 0.5 mg TPGS) was neuroprotective against ischemia/reperfusion injury in the rat. Previously, we reported that topical administration of TPGS at the same concentration did not have a neuroprotective effect *in vivo*[81]. This discrepancy is likely to the lower concentration reaching the retina compared to invasive application, typically estimated to be ~3% of the topically applied dose[37]. Although our previous work with this model suggests that administration of TPGS only did not appear to have a neuroprotective effect in its own right, a synergism between curcumin and TPGS is extremely likely, if not via the neuroprotective effects of TPGS alone, then perhaps via TPGS mediated modulation of P-gp activity, enhancing curcumin transport across ocular barriers[49,81].

The neuroprotective effect of curcumin loaded nanocarriers observed in this study may be a result of treatment commencing two days before model induction, suggesting this therapy may be most effective for patients at risk of IOP spikes such as following phacoemulsification surgery[86] or as a prophylactic to patients identified at high risk of developing glaucoma such as those with ocular hypertension or other glaucoma risk factors[2]. Furthermore, with the development of new techniques such as DARC (detection of apoptotic retinal cells) with the potential to diagnose glaucoma earlier in the disease process[87], therapies to slow or prevent RGC loss at earlier stages of disease progression will play a greater role in glaucoma management.

In conclusion, this study describes a novel nanocarrier formulation of curcumin in TPGS/Pluronic F127 that increases the solubility of this poorly soluble drug by a factor of almost 400,000. This formulation incorporates 4.3 mg/mL of curcumin with an encapsulation efficiency consistently >90% and excellent stability in liquid or lyophilized forms for at least two months when stored at room temperature, as determined by HPLC and spectroscopic techniques. This formulation was found to be neuroprotective against glutamate and cobalt chloride induced injury in retinal cultures *in vitro* and significantly preserved RGC density in two well-established rodent models of ocular injury. In conclusion, we demonstrate that curcumin loaded nanoparticles have exciting potential for overcoming ocular barriers and may facilitate the translation of curcumin based therapies to the clinic for the treatment of ocular conditions such as glaucoma.

Methods and Materials

Preparation of curcumin loaded nanocarriers. Curcumin, D-α-tocopherol polyethene glycol 1000 succinate (TPGS) and Pluronic F127 were obtained at the highest available purity from Sigma-Aldrich (Kent, UK). Curcumin-loaded nanocarriers (CN) were prepared using an adaptation of the thin-film hydration technique described previously[88]. Curcumin, TPGS, and Pluronic F127 were dissolved in ethanol to a concentration of 5 mg/mL, 10 mg/mL and 20 mg/mL respectively; with 10 min of gentle heating and bath ultrasonication to clarity. Solutions were aliquoted in the desired molar ratio (22.55 mM, 12.22 mM 7.94 mM of TPGS, curcumin, and Pluronic F127 respectively) into a round bottom flask, mixing well. The solvent was removed by rotary evaporation (50 mBar, 65 °C, 2 h) using a Rotavapor R210 with a V850 Vacuum controller (Buchi, Switzerland) while protecting from light. After this time, the thin-film was rehydrated (50 °C, 0.5 h) in the desired buffer (distilled water, phosphate buffered saline (pH 7.4) or HEPES trehalose buffer (10 mM HEPES, 50 mg/mL trehalose, pH 7.4). Unencapsulated curcumin was then removed from the formulation by 0.22 µm filtration (33 mm Millex filter, Merck Millipore, USA) as shown in Fig. 1B. Free-curcumin (FC) was prepared according to the same protocol as described above, without the addition of TPGS or Pluronic F127.

Lyophilisation of curcumin loaded nanocarriers. Lyophilisation of CN formulations in HEPES trehalose buffer was achieved by equilibrating 1 mL aliquots of nanocarriers in 7 mL screw neck squat form glass vials (CamLab, Cambridge UK) at 25 °C before freezing at −60 °C for 2 h at 760 Torr. Primary drying of samples was completed at −38 °C at 200 mTorr for 24 h, followed by a secondary drying phase at 25 °C and 200 mTorr for 2 h. Samples were capped immediately after cessation of secondary drying before storing at 25 °C while protecting from light until required. For stability assessment, samples were rehydrated for 30 minutes by addition of 1 mL of 0.22 µm filtered distilled water with gentle mixing.

The moisture content of formulations was determined using thermogravimetric analysis (TGA). Freeze dry samples were placed in an aluminium pan and analysed by a Discovery TGA (TA instruments, USA). Samples were purged with a flow rate of 25 mL/min nitrogen gas and heated from 30 to 200 °C with 10 °C/min rate. The percent mass loss was calculated by TA Instruments Trios software at 120 °C for water content. Three freeze dry formulations were measured three times for each sample.

Curcumin loading efficiency. The loading efficiency of CNs was determined spectroscopically and results confirmed using HPLC. Spectroscopic determination of curcumin loading was achieved by diluting in DMSO 1:500 at 435 nm normalised to empty nanocarriers. The concentration of curcumin in each formulation was then determined using the molar extinction coefficient of curcumin (Fig. 1) determined by constructing a standard curve measuring the absorbance of known curcumin concentrations. The encapsulation efficiency of each formulation was calculated using Eq. 1;

$$EE\% = 100 \cdot \left(\frac{[C]_E}{[C]_S} \right) \quad (1)$$

where $[C]_S$ is the concentration of curcumin originally added to the formulation (typically 4.5 mg/mL) and $[C]_E$ is the concentration of curcumin detected spectroscopically within the nanocarriers after 0.22 µm filtration to remove unencapsulated material. Results were confirmed using an adaptation of an established HPLC technique[89]. Briefly, curcumin containing samples were diluted in methanol before 20 µL volumes were injected at 25 °C onto a Phenomenex® Synergi (4 µm Polar—RP 80 A with size of 250 × 4.60 mm) column with an Acetonitrile: 0.1% trifluoroacetic acid 50:50 solvent system at a flow rate of 1 mL/min connected to a Agilent Technology 1260 Infinity HPLC system. Absorbance was recorded at 420 nm and the area under the curcumin elution curve compared to a standard curve of known curcumin concentrations.

Dynamic light scattering. Particle size was determined using a Malvern Zetasizer. Measurements of particle diameter and polydispersity index were recorded from a minimum of three formulations for each experimental condition or time point after manufacture. Nanocarriers were diluted 1 in 10 in the appropriate buffer prior to recording.

Transmission electron microscopy. Nanocarrier suspensions were processed using carbon grids to absorb particles from suspension before staining with 1% uranyl acetate for 1 min and drying. Specimens were observed using a Joel-1010 Transition Electron Microscope operated at 100 kV with images acquired using a Gatan Orius digital camera.

X-ray diffraction and FT-IR. X-ray diffraction graphs of drug alone, empty nanoparticles or CN were prepared from X-ray diffractometer (Rigaku MiniFlex 600) and the 2-thea angle was set from 5° to 65° with an angular increment of 0.05°/second. The measurements were performed at a voltage of 40 kV and 15 mA. The FT-IR spectrum of free curcumin, empty nanoparticles and CN were recorded using a PerkinElmer Spectrum 100 FT-IR spectrometer at 4 cm^{-1} resolution, with 4 scans between 4000 cm^{-1} and 650 cm^{-1}.

Curcumin release assay. *In vitro* curcumin release was assessed using an adaptation of a previously described protocol[41]. Briefly, free curcumin (dissolved in 95% ethanol) or CNs containing 4.5 mg/mL of curcumin was loaded into a 1 mL Spectra-Por Float-A-Lyzer dialysis cassette (Sigma-Aldrich) with 3.5–5 kDa molecular weight cut-off. Samples were dialysed against 200 mL of PBS containing 10% Tween-80 to act as a sink for released curcumin maintained at 37 °C with stirring at 50 rpm. At the specified time points, samples were removed from the mixture and replaced with fresh buffer. The concentration of curcumin was determined as described above. Results from three experimental replicates were fit to a single phase association (Eq. 2);

$$Y = Y0 + (\text{Plateau} - Y0) * (1 - \exp(-K * x)) \quad (2)$$

where Y_0 = zero, Plateau is the maximal release and K is the rate of curcumin release (h^{-1}) from which half-life ($t_{1/2}$) was calculated ($t_{1/2} = \ln 2/K$).

Cell culture. R28 cell line (Kerafast, Boston, MA) were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Paisley, UK) supplemented with 5% foetal bovine serum (Invitrogen, UK), 100 U/ml penicillin, 100 µg/ml of streptomycin and 0.292 mg/mL glutamine (Gibco, UK), 7.5% sterile dH20 and 1.5 mM KCl (Sigma-Aldrich, UK). The medium was changed every other day and cultures were passaged at 90% confluence.

Cell viability assessment. R28 cells were plated at 4,000 cells/well in 96-well plates for 24 h before treatment with varying concentrations of curcumin (0 to 20 µM) or an equivalent concentration of TPGS/Pluronic F127 only nanocarriers (vehicle control) in conjunction with varying concentrations of cobalt chloride or glutamate insults for a further 24 h. Cell viability was assessed in each case using the Alamarblue (Invitrogen, UK) assay according to manufacturer's instructions. Briefly, the Alamarblue solution was added to each well-plate and incubated for 4 hours before recording the fluorescence using a Safire plate reader excitation of 530 nm and emission of 590 nm[90].

Animals. All animal experiments were performed with procedures approved by the U.K. Home Office and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. For *in vivo* assessment of experiments: in total 48 Adult male Dark Agouti (DA) rats (Harlan Laboratories, UK) weighing 150 to 200 g were housed in an air-conditioned, 21 °C environment with a 12 h light-dark cycle (140–260 lux), where food and water were available *ad libitum*. 13 animals served as naïve controls which were not subject to further interventions before immunohistochemistry.

Ocular hypertension (OHT) model. Ocular hypertension was surgically induced in the left eye of 18 DA rats (5 OHT only, 5 OHT + CN, 8 OHT + FC) as described previously[91]. Procedures were conducted under general anaesthesia using a mixture of 37.5% Ketamine (Pfizer Animal Health, Exton, PA), 25% Dormitol (Pfizer Animal Health, Exton, PA) and 37.5% sterile water, at 2 mL/kg administered intraperitoneally. Briefly, 50 µL of hypertonic saline solution (1.8 M) was injected into the two episcleral veins using a syringe pump (50 µL/min; UMP2; World Precision Instruments, Sarasota, FL, USA). A propylene ring with a 1 mm gap cut from the circumference was placed around the equator to prevent injected saline outflow from other aqueous veins. The IOP from both eyes of each rat was measured at regular intervals using a TonoLab tonometer (Tiolat Oy, Helsinki, Finland) under inhalational anaesthesia (0.4% isoflurane in oxygen). Daily administration of topical CNs was performed

in 5 DA rats (two 35 µL drops/day 5 min apart at 10 am each day) starting two days prior to model induction and continuing until model termination (21 days post IOP elevation) with 5 rats serving as OHT only controls. An additional 8 rats received free-curcumin (FC) prepared using the same protocol as CN curcumin without the addition of TPGS or Pluronic F127. FC was administered to OHT animals using the same dosing regime as described for CN. Animals were sacrificed three weeks after unilateral IOP elevation and retinas flat-mounted prior to Brn3a immunohistochemistry.

Partial optic nerve transection (PONT) model. Partial optic nerve transection was conducted in the left eye of 17 DA rats, using a previously described technique[92]. Under general anaesthesia, an incision was made in the superior conjunctiva, and the ON sheath was exposed. A longitudinal slit was next formed in the dura mater to expose the ON and a 0.2-mm cut was made in the dorsal ON, 2 mm behind the eye using an ophthalmic scalpel with steel cutting guard. Damage to major ophthalmic blood vessels was avoided and verified at the completion of surgery by ophthalmoscopy. Daily administration of topical CNs was conducted in 9 DA rats after induction of the pONT model using the same treatment regimen as described previously with the remaining 8 serving as pONT only controls.

Brn-3a immunohistochemistry and confocal microscopy. Brn-3a labelling of RGCs in retinal whole-mounts was completed as described previously[57]. Briefly, eyes were enucleated upon sacrifice and fixed in 4% paraformaldehyde at 4 °C overnight before dissecting retinal whole mounts. Whole mounts were stained for the RGC specific nuclear-localised transcription factor Brn3a using an anti-mouse mAb (1:500, Merck Millipore, Darmstadt, Germany) and examined under confocal microscopy (LSM 710, Carl Zeiss MicroImaging GmbH, Jena, Germany). Each retinal whole mount was imaged as a tiled z-stack at x10 magnification which was used to generate a single plane maximum projection of the RGC layer in each retina for subsequent analysis. Each whole mount image was manually orientated so that the superior retina was towards the top of the image using *in vivo* cSLO imaging of retinal vasculature as a reference. Retinal image acquisition settings were kept constant for all retinas imaged, allowing comparison of Brn3a expression in each experimental group as previously described[93]. Automated quantification of Brn3a labelled RGCs in retinal whole-mounts was completed as described previously[57].

Statistical Analysis. All data were analysed with the Student's *t*-test, ANOVA or with appropriate post hoc testing using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) as appropriate. Data were presented as means ± SE and $p < 0.05$ was considered significant. Molecular structures were drawn using ACD/ChemSketch 2015 and all images were taken by the authors (BMD).

Data availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

B.M.D., S.S. and M.F.C. designed the research. B.M.D., M.P., S.B., P.S., E.S., H.H., N.R., G.M., C.S., L.G., A.N. and A.S. performed experiments, B.M.D., M.P. and M.F.C. wrote the paper. All authors read and commented on the manuscript.

Additional Information

Competing Interests: M.F.C., B.M.D. and S.S. have a patent application pertaining to the nanoparticle curcumin formulation described in this study. The authors declare no additional competing interests.

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3. Diskussion

Das Glaukom, definiert als progressive Optikusneuropathie mit Verlust retinaler Ganglienzellen und ihrer Nervenfasern, spielt eine wichtige sozioökonomische Rolle bei einer globalen Prävalenz von aktuell 3,54% (ungefähr 80 Millionen Betroffene, Bevölkerungsschicht 40-80 Jahre)[1,3,4,14]. Hochrechnungen zufolge steigt die Zahl auf 111,8 Millionen Erkrankte weltweit im Jahre 2040[14]. Risikofaktoren für ein POWG, abgesehen von okulären Strukturen, ist das männliche Geschlecht, refraktive Fehler, Leben in der Stadt und ein afrikanischer Ursprung/Verwandtschaftsgrad[7,14].

Das wichtigste Ziel ist die frühzeitige Diagnosestellung eines glaukomatösen Schadens und der rechtzeitige Beginn einer individuell adaptierten Therapie. Die Diagnosestellung basiert auf den Pfeilern der Tonometrie, Funduskopie, Perimetrie und komplexer bildgebender Verfahren (OCT, HRT, Fundusphotographie) zur Darstellung der Ganglienzellschicht und ihrer Axone, der Photorezeptoren, und der anterioren Strukturen des Nervus Optikus. Die Therapie eines Glaukoms gliedert sich in zwei große Bereiche, die medikamentöse Behandlung und operative Verfahren, die in einem Stufenschema den individuellen Bedürfnissen des Patienten angepasst werden.

Die dieser Habilitationsschrift zugrundeliegenden Originalarbeiten sollen den aktuellen Versorgungsansatz des Glaukoms darlegen. Insbesondere die Einsatzmöglichkeiten der chirurgischen und neuroprotektiven Therapien in der klinischen Anwendung wurden dabei evaluiert. Die vorgestellten Studien werden in den folgenden Kapiteln zusammenfassend diskutiert.

3.1. Operative Verfahren zur Einstellung des Augeninnendruckes

Die operativen Therapieverfahren werden hauptsächlich nach Ausnutzung aller möglichen medikamentösen Optionen oder nach komplikativer, individuell nicht akzeptierter medikamentöser Therapie angewandt.

Die mikro-invasiven Operationsverfahren (Trabectome®, iStent inject®) bieten einen gewebeschonenden Ansatz für aufbauende, glaukomchirurgische Eingriffe im Krankheitsverlauf, eine effektive Drucksenkung bei mildem bis moderatem primären und sekundären Offenwinkelglaukom und eine Reduktion der Glaukommedikation[19,23,36,40,41].

Die Elektroablation des Trabectome® Verfahrens führt zu einer Shuntverbindung für den Kammerwasserfluss von der Vorderkammer in den Schlemm'schen Kanal unter Umgehung des intratrabekulären Widerstands. Nach aktueller Studienlage führt die Trabectome® Operation zu einer Reduktion des IOD um 6-10 mmHg bei POWG[20,30–33]. Wir berichten übereinstimmend eine Drucksenkung im unteren Bereich von etwa 5,0 mmHg ein Jahr postoperativ[36]. Bei Betrachtung eines erweiterten Zeitraumes über drei Jahre bleibt der IOD auf dem Niveau der 1-Jahreswerte[19]. Vergleichsstudien erwähnen eine Senkung der Medikation um 0,9-1,2 Medikamente[32,33]. Die Ergebnisse finden sich im Klinikalltag nicht ganz wieder; unsere Patienten stellen sich mit reduzierter Glaukomtherapie um 0,7 Medikamente drei Jahre postoperativ vor[19,36].
Der Pathogenese des PEX-Glaukoms folgend (Ablagerung eines extrazellullären, fibrillären Materials im Trabekelmaschenwerk) wurde eine bedeutende Drucksenkung durch die Trabectome® Operation verglichen zu dem POWG erwartet und kann durch unsere Arbeiten und internationale Kollegen mit einer Senkung des IOD um 7-12 mmHg bis zu drei Jahre postoperativ bestätigt werden[19,20,33,36]. In unserer Studie liegt der Durchschnitt der Therapiereduktion, ähnlich der Vergleichsstudien, um 0,6 Medikamente nach drei Jahren[19,33].

Bei Betrachtung der Einflussgröße Kataraktoperation kann eine moderat stärkere Drucksenkung in der kombinierten Trabectome® + Katarakt Kohorte nachgewiesen werden[20,33]. Die Indikationsstellung zur kombinierten Operation schließt weitere Parameter als nur den drucksenkenden Effekt ein. So steht vor allem eine Sehverschlechterung der Patienten und bei aus diesem Grund anstehender Kataraktoperation auch die Reduktion der medikamentösen Glaukomtherapie im Vordergrund, so dass bei tieferen Basis-IOD-Werten eine Intervention als bei einem alleinigen glaukomchirurgischen Eingriff durchgeführt wird[20,33]. In unserem Kollektiv können wir über eine deutlichere Drucksenkung im POWG mit kombinierter Trabectome® + Katarakt Operation (p=0,017) berichten[36]. Dagegen wurde der IOD im PEX-Glaukom nicht verstärkt durch den kombinierten Eingriff gesenkt[36]. Weiter untersuchten Minckler und Kollegen 1127 Patienten aus der Trabectome® Study Group sowohl nach alleiniger als auch kombinierter Trabectome® Operation[93]. Nach 24 Monaten konnte der IOD um 40% von 25,7 mmHg auf 16,6 mmHg in der solitären Trabectome® Gruppe reduziert werden im Vergleich zu der kombinierten Trabectome®+ Katarakt Gruppe, die eine IOD Senkung um nur 30% von 20,0 mmHg auf 14,9 mmHg aufzeigt[93]. Man erkennt hier deutlich, dass in dem Kombinationseingriff der drucksenkende Effekt nicht allein zur Indikationsstellung

herangezogen wurde und bei geringerem Ausgangsdruck operiert wurde als in der alleinigen Trabectome® Gruppe[93].

Eine vergleichbare Drucksenkung um ungefähr 6 mmHG ist auch mittels der modifizierten Goniotomie in der POWG und PEX-Glaukom Kohorte ein Jahr postoperativ zu erreichen[45]. Die Medikation konnte um die Anzahl der Medikamente 1,1 in POWG und um 0,7 im PEX-Glaukom reduziert werden[45]. Die Daten entsprechen den Studienergebnissen in MIGS[19,20,30,31,33]. Auffällig ist eine leicht verminderte IOD Reduktion im PEX-Glaukom durch die Goniotomie im Vergleich zu den Durchschnittswerten der anderen MIGS Verfahren. Direkte Referenzdaten zu der modifizierten Goniotomie liegen unserem Wissen nach nicht vor. Die Trabectome® und Goniotomie Operation zeigt keine signifikante Differenz in Bezug auf die Parameter IOD und Medikation[45].

Die weitere Diskussion schliesst die Erfahrungen mit dem iStent inject® ein: Die iStent inject® Implantation kreiert einen direkten Anschluss der Vorderkammer an den Schlemm'schen Kanal unter Umgehung der Stelle des höchsten Widerstandes für den Abfluss des Kammerwassers – das juxtakanalikuläre Trabekelmaschenwerk[94].

Katz und Kollegen untersuchten den Effekt der singulären versus zwei oder drei iStent inject® Implantationen in POWG, PEX-Glaukom und Pigmentdispersionsglaukom über 18 Monate[95]. Die Ergebnisse wurden nicht in die verschiedenen Glaukomtypen unterteilt. Nach 18 Monaten konnte der IOD auf 15,9 mmHg in der 1-Stent Kohorte, auf 14,1 mmHg in der 2-Stent-Gruppe und auf 12,2 mmHg in der 3-Stent-Gruppe reduziert werden[95]. Die IOD Reduktion war signifikant höher mit der Implantation jedes zusätzlichen Stents um 1,8 mmHg für die 3-Stent versus 2-Stent-Kohorte und um 1,7 mmHg für die 2-Stent versus die 1-Stent-Gruppe ($p<0{,}001$)[95]. Daraus folgernd wird die 2. Generation, der iStent inject®, als zweifacher Stent in das Trabekelmaschenwerk implantiert.

Die Indikationsstellung ist allen MIGS Verfahren ähnlich; ein wichtiger Unterschied zu der Trabectome® und Goniotomie Operation bleibt das Persistieren eines Titanobjekts in dem Körper des Patienten.

In einer phaken Offenwinkelglaukom Kohorte erreichen 66% der Probanden einen IOD ≤ 18 mmHg ohne Medikation und 81% der Probanden einen IOD ≤ 18 mmHg mit entweder einem Medikament oder ohne Medikamente nach 12 Monaten einer iStent® Implantation[40]. Der durchschnittliche Ausgangswert verringert sich um 39,7% von 26,3 ± 3,5 mmHg auf 15,7 ± 3,7 mmHg nach einem Jahr[40]. Auch PEX-Glaukom und Pigmentdispersionsglaukom wurden ohne Evaluation der Ergebnisse in den unterschiedlichen Glaukomsubtypen in die Studie

eingeschlossen[40]. In unserer Studie zeigt sich eine übereinstimmende Drucksenkung um 33% auf 14,2 mmHg im POWG und um 35% auf 15,3 mmHg im PEX Glaukom[41]. Auch Arriola-Villalobos et al. demonstrieren eine ähnliche IOD Reduktion um 35,7% in der Kombination Katarakt und iStent inject® Operation nach einem Jahr[39].

Eine Reduktion präoperativer Medikation erreichen 86,9% der Patienten, einschließlich 15,2% mit einer Reduktion von einem Medikament und 71,7% mit einer Reduktion von zwei oder mehreren Medikamenten[40]. In unserer Studienkohorte wird die Anzahl der Medikamente um 1,3 im POWG und um 1,2 im PEX Glaukom gesenkt[41].

In unserer Arbeit kann eine postoperative persistierende IOD Steigung in Patienten mit Pigmentdispersionsglaukom nach iStent inject® Implantation beobachtet werden, so dass größere Fallzahlen den limitierenden Effekt bezüglich eines Pigmentdispersionsglaukoms evaluieren müssen[41].

Komplikative Ereignisse während oder nach einer MIGS Chirurgie werden hier zusammenfassend erörtert. In fast allen vorherigen Studien wird ein intraoperatives Hyphäma nach iStent inject® und Trabectome® Operationen genannt. Es entsteht durch Blutreflux aus dem eröffnetem Schlemm'schen Kanal und den Collector channels. Es kann auch als direkte Bestätigung des Operationserfolgs und Eröffnen der richtigen anatomischen Strukturen gewertet werden[20,33,36,41,93]. Weiter wurde in Vergleichstudien über postoperative IOD Spitzen, meistens direkt an dem darauffolgenden Tag berichtet[96]. Dies konnten wir in unseren Arbeiten nicht beobachten[19]. Weiter ist die Ausbildung peripherer anteriorer Synechien und ein Membranwachstum nach Fibrinreaktion bekannt. Dies kann zu einem Verschluss der Stent Öffnung führen[96]. In unserem Kollektiv aus dem klinischen Alltag konnte kein Stent Verschluss durch Membranwachstum nachgewiesen werden[41]. Die Inzidenz der iStent inject® Obstruktion und Fehlstellung wird mit <5% aller Fälle angegeben[40]. Nach einem Jahr Nachbeobachtungszeitraum nach iStent inject® wurden <5% aller Fälle bei IOD Dekompensation einer erneuten medikamentösen Therapie oder einem chirurgischen Eingriff in Form einer Trabekulektomie, Goniotrepanation oder tiefen Sklerektomie zugeführt[40]. 14% nach solitärer Trabectome® Operation benötigten anschließend weitere glaukomchirurgische oder laserchirurgische Eingriffe[93]. 5,9% aller 1127 Patienten wurden in dem Zwei-Jahres-Zeitraum einer anschließenden Trabekulektomie zugeführt[93]. In einer anderen Studie fällt ein verzögerter Beginn eines Hyphämas mit schwankendem Visus im Durchschnitt nach 8,6 Monaten postoperativ auf (range 2-31 Monate)[97]. Dieses Phänomen ist generell selten (4,6%, 12 von 262 Patienten)[97]. Risikofaktoren für das Auftreten ist das Valsalva Manöver, die

Einnahme blutverdünnender Medikamente und ein IOD unter dem episkleralen venösen Druck mit physiologischem Blutreflux in die Vorderkammer, da das Trabekelmaschenwerk entfernt oder umgegangen wurde und ein direkter Zugangsweg zum Schlemm'schen Kanal existiert[97].
Die Vorteile der MIGS lassen sich durch ihr hohes Sicherheitsprofil, konjunktivasparenden Ansatz, komplikationsarme postoperative Nachsorge, Reduktion der antiglaukomatösen Therapie und effektive Drucksenkung auf Werte um 15mmHg zusammenfassen[20,31,33,36,41,93]. Zukünftige penetrierende Glaukomeingriffe können durch Vermeidung externer Narbenbildung bei einem ab interno Zugang komplikationsarm durchgeführt werden[20,31,33,36,41,93]. Die normale Architektur und physiologische Anatomie des Auges wird nur minimal durch den chirurgischen Eingriff beeinflusst. Die MIGS Verfahren benutzen biokompatible Stoffe und unterstützen vorwiegend die physiologischen Abflusswege des Kammerwassers.
Es wurde kein Auftreten von Komplikationen überwiegend assoziiert mit filtrierender Glaukomchirurgie wie eine Endophthalmitis, persistierende Hypotonie, choroidales Effusionssyndrom, Blebitis und suprachoroidale Hämorrhagie in MIGS berichtet[20,31,33,36,41,93,98]. Ein weiterer Vorteil stellt die Kombinationsmöglichkeit mit anderen intraokularen Eingriffen beispielsweise der Kataraktchirurgie dar[36,93].
Durch die schnelle Erholungsphase postoperativ bleibt die Lebensqualität der Patienten auf hohem Niveau erhalten[18].
Allerdings ist der wichtigste Faktor für die Indikation eines glaukomchirurgischen Eingriffs das Niveau der Drucksenkung, so dass bei Zielwerten unter 12mmHg die TE die Operation der Wahl vieler Operateure ist[99]. Die IOD Reduktion der MIGS Verfahren ist limitiert auf Werte um die 15mmHg, so dass die Techniken nicht bei fortgeschrittenem Glaukom eingesetzt werden können, die eine deutlich stärkere Drucksenkung zur Stabilisierung des Restgesichtsfeldes und Visus verlangen[98]. Dieser Kompromiss in Wirksamkeit ist allerdings durch das geringe Risikoprofil der MIGS ausgeglichen. Im Gegensatz zu MIGS kann die TE eine langfristige Drucksenkung auf 8-12mmHg erreichen, auch ohne additive Medikation[53].
Die Entscheidung, welche chirurgische Technik für den individuellen Patienten angemessen erscheint, sollte unter Beachtung der einzelnen Parameter der Lebensqualität, des Augeninnendrucks und der Glaukomtherapie getroffen werden. Es wurde kein Unterschied in unserer vorliegenden Arbeit in Bezug auf die Lebensqualität zwischen der TE und den minimal-invasiven, glaukomchirurgischen Verfahren gefunden[18]. Wie erwartet präsentiert sich die Drucksenkung deutlich stärker in der TE Kohorte im Vergleich zu MIGS[18]. Die Anzahl der

Medikamente wurde auch durch die TE stärker gesenkt im Vergleich zu MIGS ($p<0,001$)[18]. Die geringere Einnahme antiglaukomatöser Medikamente wirkt sich auf die Lebensqualität aus, auch wenn die Glaukomtherapie nicht Gegenstand des NEI-VFQ-25 Fragebogens ist[18]. Allerdings erfordert die TE innerhalb der ersten 6 Monate signifikant mehr postoperative Nachkontrollen (9) als beispielsweise die iStent inject® Implantation (3)[99].

Neben den primären Glaukomarten spielen die inflammatorischen Sekundärglaukome eine wichtige Rolle im klinischen Alltag. Häufig beginnt die Therapieeinstellung aus einer akuten Entzündungssituation heraus - mit langwierigen Verlaufskontrollen aufgrund der primär schwierigen Ausgangssituation. Häufiger Grund einer anterioren Uveitis ist die virale Genese mit einer Cytomegalie-, Herpes simplex-, Varizella zoster- oder Rubella- Virus Infektion[24]. Auch hier liegt die Empfehlung klinischer Leitlinien (European Glaucoma Society EGS Guidelines 4th Ed.) primär in der Druckreduktion zum Erhalt der RGC und ihrer Axone. In unserem großen Kollektiv (n=270) konnten wir erstmalig in diesen verschiedenen Virusgruppen die aktuellen chirurgischen Therapiemöglichkeiten analysieren[24]. Die TE bleibt als Goldstandard zur zügigen und ausgeprägten Druckreduktion auf Druckwerte um die 10mmHG erhalten[24]. In Vergleichsstudien von Shimizu und Iwao et al. wird das Vorgehen bestätigt[100,101]. Der Stellenwert der MIGS in Form einer iStent® inject und Trabectome® Operation lässt sich in seiner komplikationsarmen, kurzzeitigen IOD Senkung sehen. In akuten Entzündungssituationen mit hohen Ausgangsdruckwerten >30 mmHg bietet die MIGS eine sichere Alternative, um schwerwiegende Komplikationen einer TE zu vermeiden und dennoch die Möglichkeit einer nachfolgenden, filtrierenden Operation offen zu halten[24]. Die individuelle Therapieentscheidung bei Vorliegen eines uveitischen Sekundärglaukoms erfordert ein Expertenteam aus Glaukomspezialist und Uveitistalent.

3.2. Neuroprotektive Wirkstoffe zur Behandlung des Glaukoms

In früheren Zeiten setzte sich die Glaukomdiagnostik neben der morphologischen Beurteilung der Retina und des Sehnervenkopfes vor allem aus der Tonometrie zusammen, da bekannterweise die Drucksenkung die anzustrebende Zielgröße ist. Weltweit etablierter Goldstandard ist die Goldmann Applanationstonometrie[17]. Auch heute nimmt man an, dass der RGC-Untergang durch einen hohen Augeninnendruck mit mechanischer Belastung der inneren Retina und der Lamina cribosa beschleunigt wird. Der Pathomechanismus erklärt sich aus

eintretender Ischämie, Gliazellaktivierung, Störung des axoplasmatischen Flusses sowie ein Neutrophinmangel mit der Folge einer RGC Apoptose[102].
Ätiologisch spielen neben der IOD-Erhöhung weitere Risikofaktoren wie oxidativer Stress, welcher durch reaktive Sauerstoff- und Stickstoffspezies induziert wird[103–106], eine verringerte retinale Perfusion[107] sowie die Glutamat-induzierte Exzitoxizität[108,109] eine Rolle. Daher kann eine Progression der glaukomatösen Optikusneuropathie trotz erfolgreicher IOD-Kontrolle eintreten. Kass et al. beobachtete bei 4,4% von insgesamt 1636 Patienten mit OHT einen chronisch progredienten Verlauf mit Entwicklung eines POWG bei zufriedenstellender Drucksituation[60]. Aufgrund dieser Tatsache werden zusätzlich zur IOD Reduktion komplementäre therapeutische Ansätze untersucht, die unter anderem darauf abzielen, den oxidativen Stresslevel und damit den apoptotischen Untergang der RGZ zu verhindern und neuroprotektive Signalwege zu aktivieren. Eine gezielte Inhibition von oxidativem Stress beispielsweise lässt sich unter anderem durch die Kontrolle endogener enzymatischer und nicht-enzymatischer Antioxidantien, Gabe von externen Antioxidantien, Steuerung der reaktiven Sauerstoffradikal - Bildung sowie Regulation der Expression von anti-oxidativen Faktoren oder Genen erreichen[110].

Zwei Neuroprotektiva stehen aktuell im Fokus der Aufmerksamkeit, Curcumin und Coenzym Q10.

Curcumin, ein pflanzliches Polyphenol, weist neben der anti-oxidativen Fähigkeit verschiedene weitere anti-inflammatorische und anti-Protein-aggregierende Charakteristika auf[62–66]. So berichten Yue et al., dass BV-2 Mikroglia, welche mit Curcumin behandelt wurden, nach der 24 Stunden-Exposition mit H_2O_2 eine signifikant höhere Zellviabilität und verminderte Apoptoserate sowie signifikant niedrigere intrazelluläre Sauerstoffradikale im Vergleich zu der Kontrollgruppe aufweisen[70]. Dieser Schutzeffekt lässt sich ebenfalls in vivo an Wistar-Ratten mit einer Curcumin Therapie über sechs Wochen beobachten[70]. Parada et al. informieren in einer Vergleichsstudie ebenfalls über eine reduzierte Apoptose in Mikroglia durch Curcumin in einem Modell (Rotenone/Oligomycin A), das zu einem Absterben der Hälfte der Mikroglia der Kontrollgruppe durch oxidativen Stress und pro-inflammatorische Kaskaden führt[111].
In einem Diabetes -Rattenmodell zeigt die Gabe von Curcumin einen protektiven Einfluss auf die äußere nukläre -, innere nukleäre - und Ganglionzellschicht[112]. Die erhöhte Glutamat Konzentration und damit einhergehende induzierte Apoptose in dem Diabetesmodell kann signifikant durch die Curcumin Gabe reduziert werden[112].

In H_2O_2-exponierten Zellen des Trabekelmaschenwerks von Schweineaugen führt Curcumin zur Reduktion der reaktiven Sauerstoffspezies-Produktion, zur Hemmung von pro-inflammatorischen Faktoren wie Interleukin (IL)-6, Endothel-Selektin (ELAM-1), IL-1α, IL-8 sowie zur Abnahme der Apoptoserate[113]. Dies geschieht unter anderem durch die Aktivierung des Nrf2-Keap1-Signalweges[114]. In der Zellkultur humaner, gegenüber H_2O_2-exponierter Zellen des Trabekelmaschenwerks reduziert die Gabe von einem Chitosan-Hydrogel, welches Latanoprost und Curcumin-beladene Nanopartikeln beinhaltet, die Genexpression von dem Tumornekrosefaktor, ICL-1α (Bifunctional glyoxylate cycle protein), IL-6 und der Matrixmetaloproteinase 13, die Apoptoserate sowie die ROS-Bildung. Bezüglich der Biokompatibilität des topisch applizierten Hydrogels können in Kaninchenaugen keine negativen Effekte festgestellt werden[115].

In unseren experimentellen Untersuchungen und in vivo an etablierten Tiermodellen mit OHT und partieller Sehnervdurchtrennung konnte gezeigt werden, dass mit Curcumin beladene Nanopartikel die retinalen Ganglienzellen vor Glutamat-induzierter Toxizität und Hypoxie schützen[67,68].

Als zweite potenziell neuroprotektiv wirksame Substanz im Glaukom ist das Coenzym Q10 zu nennen. In experimentellen Studien basierend auf einer Zellkultur und verschiedenen Tiermodellen wurde gezeigt, dass CoQ10 die RGC und die den Sehnerv umgebenden Astrozyten vor oxidativem Stress schützt und Ganglienzellschäden ausgelöst durch IOD - Anstiege, Exzitotoxizität oder durch strahleninduzierte Apoptose verhindert[90–92,116–118]. Lee und Kollegen konnten nachweisen, dass CoQ10 das RGC Überleben um 30% erhöht, die axonale Gesundheit des Sehnervens bewahrt sowie die astrogliale Aktivierung durch Verringerung der GFAP-Expression (Glial fibrillary acidic protein, saures Gliafaserprotein) in der Retina und in dem Sehnerv in einem Glaukom Mausmodell (DBA/2J) inhibiert[89]. CoQ10 reduziert die Apoptoserate der RCG durch Herabsetzen der Bax-Proteinexpression oder auch Hochregulierung der pBad-Proteinexpression[89]. Hinzuzufügen ist, dass CoQ10 den mitochondrialen DNA-Gehalt und die Komplex-IV-Proteinexpression in der Netzhaut von glaukomatösen DBA/2J-Mäusen erhalten konnte[89].

In einer klinischen Studie untersuchten Parisi et al. die Funktion der Netzhaut mittels eines Muster-Elektroretinogramms (PERG) und die Nervenfunktion in der Sehbahn mittels visueller evozierter Potenziale (VEP) im POWG, die in einer Gruppe zusätzlich zu der ß-Blocker-Monotherapie mit einer topischen Gabe von CoQ10 behandelt wurden[119]. Es wurde festgestellt, dass die Parameter PERG und VEP 6 und 12 Monaten nach der Behandlung in der

Kontrollgruppe unverändert bleiben, während in der CoQ10 Gruppe bezüglich PERG P50 und VEP P100 eine verkürzte Gipfelzeit und bezüglich PERG P50-N95 und VEP N75-P100 eine Zunahme der Amplitude gemessen wurden[119]. Die Forschungsgruppe sieht einen Nutzen durch die Einnahme von CoQ10 im POWG auf die Funktion der visuellen Neurone und ihrer Axone[119].

Sowohl Curcumin als auch CoQ10, welche topisch zweimal täglich für drei Wochen verabreicht wurden, konnten in unseren Rattenmodellen (OHT/partielle Sehnervdurchtrennung) eine neuroprotektive Wirkung auf die retinalen Ganglienzellen zeigen[67,68].

Es gilt, diese Therapiestudien von Tiermodellen auf Patienten zu translatieren. Dieser große Schritt wurde durch Prof. Cordeiro eingeleitet, in dem Sie zuerst das Diagnoseverfahren DARC (Detektion apoptotischer retinaler Ganglienzellen) etabliert hat, welches eine kurzzeitige Verlaufskontrolle einer RGC Apoptose in vivo am Menschen ermöglicht[120]. Ohne zeitnahe Monitoring Möglichkeiten ist ein Therapieeffekt neuroprotektiver Medikamente sehr schwer nachzuweisen. Ein Studiendesign mit beispielsweise drei- bis fünfjähriger Medikamenteneinnahme ist ethisch schwer zu rechtfertigen, selbst wenn erst nach diesem Zeitraum genaue Aussagen zu Gesichtsfelddefekten, Verlauf der retinalen Nervenfaserschichtdicke und Visus im POWG zu erwarten sind[121,122]. Diese Limitationen müssen überwunden werden, um einen zugelassenen Medikamentenstatus und eine Kostenübernahme durch die Krankenkassen zu erreichen. Aktuell werden die Wirkstoffe häufig als Nahrungsergänzungsmittel im Internet- oder Apothekenhandel verkauft.

Abschließend bieten neuroprotektive Wirkstoffe ein enormes Potential in der Therapie neurodegenerativer Erkrankungen, die histopathologisch und biochemisch alle verschiedenen Gemeinsamkeiten teilen. Zur Auswahl des richtigen Neuroprotektivums müssen Begleiterkrankungen des Patienten beachtet werden und dementsprechend ein individueller Ansatz verfolgt werden, da neuroprotektive Wirkstoffe auf verschiedenste Signalkaskaden und Organsysteme Einfluss nehmen. Wichtig ist auch, dass die Wirkungsmechanismen der neuroprotektiven Therapie für den Glaukomexperten verständlich bleiben, um die richtige individuelle Therapie – chirurgisch oder medikamentös - für einen Patienten einleiten zu können. Aktuell stehen mit Curcumin und Coenzym Q10 zwei der interessantesten Neuroprotektiva im Vordergrund, die sowohl in vielen neurodegenerativen Erkrankungen wie Morbus Alzheimer, Morbus Parkinson, Glaukom und Apoplex, als auch in verschiedenen

Tiermodellen mit Fokus auf die neurodegenaritve Pathogenese signifikante, positive Effekte auf das neuronale Überleben zeigen konnten[63,67,71–73,89].

4. Zusammenfassung

Die Entscheidung von der Diagnosestellung über Verlaufskontrollen zur Therapieauswahl stellt gerade bei Glaukompatienten eine Schlüsselrolle dar. Da kein einziges Verfahren zur schnellen Diagnosesicherung existiert, muss unter Beachtung verschiedener, technisch komplexer Parameter die aufwendige Diagnosestellung und Therapieentscheidung durch einen Glaukomexperten erfolgen. Die aktuell wichtigsten bildgebenden Verfahren sind die optische Kohärenztomographie, Heidelberg Retina Tomographie und die Fundusphotographie[123–130]. Die verschiedenen Parameter dieser Techniken bilden den vorhandenen glaukomatösen Schaden ab und unterstützen die Therapieentscheidung nach Diagnosestellung und Verlaufsbeurteilung des Augenarztes maßgeblich[123–128,130].

Die Diagnose Glaukom führt zur Einleitung therapeutischer Schritte; im Regelfall Beginn einer antiglaukomatösen Tropfenapplikation oder einer chirurgischen Intervention. Auch eine weitere Verlaufskontrolle ist eine aktiv getroffene Entscheidung. Die klinischen Leitlinien (European Glaucoma Society EGS Guidelines 4th Ed.) empfehlen aktuell Therapieansätze zur IOD Reduktion als wichtigstem Risikofaktor der Glaukom- Progression.

Mit der Entwicklung minimal-invasiver Operationsverfahren kann eine zusätzliche Möglichkeit zur effektiven Drucksenkung mit Erhalt der Lebensqualität in milden bis moderaten primären und sekundären Offenwinkelglaukomen den Patienten angeboten werden[18,19]. Sie können dem Patienten sowohl als nächster Schritt bei insuffizienter Tropfentherapie als auch bei aus persönlichen Gründen abgelehnter antiglaukomatöser Therapie offeriert werden, bevor bulbuseröffnende, filtriende Verfahren eingesetzt werden müssen. Weiter stehen sie auch als drucksenkende Methode bei nicht erfolgreicher penetrierender Chirurgie zur Verfügung[129].

Im Vergleich zu der TE stellt die MIGS eine komplikationsarme Methode in der operativen Glaukomtherapie dar, der dadurch die limitierte Drucksenkung auf Werte in dem mittleren Zehnerbereich ausgleicht[19,20,31,33,36,41,93]. Der hohe Stellenwert der TE bis heute ist vor allem auf die Effizienz der IOD Reduktion und Einsparen einer Glaukomtherapie zurückzuführen[18,53,99].

Zusätzlich rückt ein neuroprotektiver Therapieansatz immer stärker in den Vordergrund. In den letzten 25 Jahren wurde noch einmal systematisch aufgearbeitet, dass das Glaukom als eine neurodegenerative Erkrankung durch Apoptose der retinalen Ganglienzellen und deren Axone zu definieren ist[3,4,59]. Es liegt sowohl eine neuronale Degeneration im visuellen Signalweg als auch eine zerebrale diffuse Diffusionsstörung vor[77,78]. Eine rein drucksenkende Therapie ist daher nicht immer imstande, die Progression eines Sehnervschadens langfristig aufzuhalten.
Neuroprotektive Wirkstoffe entstammen nicht einer biochemischen Familie, sondern sind Faktoren aus unterschiedlichen Signalkaskaden mit anti-oxidativen, anti-inflammatorischen und anti-Protein aggregierenden Eigenschaften. Beispielsweise gehören dazu Glutamat-Antagonisten, Gingko biloba Extrakt, Brimonidin, pflanzliche Polyphenole wie Curcumin und Resveratrol, Kalziumkanalblocker, CoQ10 und NOS-Inhibitoren[131]. Die Wirkung entfalten neuroprotektive Substanzen unabhängig von dem okulären Risikofaktor IOD[67,68,131].
Vielseitige Einsatzmöglichkeiten ergeben sich durch Nutzung der vielfältigen Signalkaskaden und die Idee einer individuell gestalteten Glaukomtherapie rückt durch Neuroprotektiva näher. Trotzallem konnte sich bisher kein neuroprotektiver Wirkstoff in klinischen Studien am Menschen durchsetzen. Hier ist vor allem das Therapie Monitoring als Hürde zu nennen, um kurzfristig retinale und neuronale Änderungen nachweisen zu können. Vielversprechende Ergebnisse der in vivo Tiermodellstudien zeigen eine signifikante neuroprotektive Wirkung von Curcumin und Coq10 auf die RGC[67,68,90,91]. Daher erfolgt aktuell der Einsatz neuroprotektiver Wirkstoffe wie Coenzym Q10 oder Curcumin als begleitende Therapie bei rascher Progression, fortgeschrittener Optikusneuropathie und schwer einzustellenden Drucksituationen. Als Applikationsformen stehen topische oder orale Lösungen bereit.
Zusammenfassend steckt der Einsatz neuroprotektiver Wirkstoffe in den Kinderschuhen. Trotz vieler in vivo Tiermodell-Studien erleben wir erst den Beginn der Translation der Neuroprotektiva auf den Menschen.

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7. Eidesstattliche Erklärung

Erklärung
§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

…..... …..

Berlin, Datum Dr. med. Milena Pahlitzsch