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Exciting Directions in Glaucoma

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Abstract

Glaucoma is a complex, life-long disease that requires an individualized, multifaceted approach to treatment. Most patients will be started on topical ocular hypotensive eyedrop therapy and over time, multiple classes of drugs will be needed to control their intraocular pressure (IOP). The search for drugs with novel mechanisms of action, to treat those who do not achieve adequate IOP control with, or become refractory to, current therapeutics, is ongoing, as is the search for more efficient, targeted drug delivery methods. Gene transfer and stem cell applications for glaucoma therapeutics are moving forward. Advances in imaging technologies improve our understanding of glaucoma pathophysiology and enable more refined patient evaluation and monitoring, improving patient outcomes.

New Glaucoma Drugs in the Pipeline

Targeting the Trabecular Meshwork

Current glaucoma therapeutics lower IOP by reducing aqueous humor formation or increasing outflow of fluid through the uveoscleral pathway. A novel strategy is targeting the trabecular meshwork cytoskeleton aiming to increase fluid outflow through the trabecular meshwork (TM) /conventional outflow pathway. (1, 2) There are several targets for this approach: 1) TM – cytoskeleton-actin microfilament disruption using marine macrolides such as latrunculins (Lat-A/B) (WARF) (3–9) (FIG 1), swinholide A, jasplakinolide (10) (WARF); 2) Protein kinase inhibition using serine–threonine kinase inhibitors such as H-7 (WARF) (11), myosin light chain kinase inhibitor ML-7 (12) and rho kinase inhibitors including Y-39983/SNJ-1656/RKI-983 (Senju / Novartis) (13–15), AR-12286 (Aerie) (16,17), AR-13324 (Aerie) (18), PG324 (which is AR-13324 combined with latanoprost) (Aerie), K-115 (Kowa) (19,20), AMA0076 (Amakem) (21); 3) targeting actomyosin contractility using nonmuscle caldesmon (WARF) (22,23) or focal adhesions

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and cell-cell adhesions with exoenzyme C3 transferase (C3) (WARF) (24). A number of these compounds are moving through clinical trials with a mechanism of action that features relaxation of the TM, expansion of juxtacanlicular spaces (JCT), dilation of Schlemm's canal SC and inhibition of actomyosin contractility. Although they comprise different classes, many of the above compounds can be effective at increasing conventional outflow since the real target is perturbing the overall system contractility, cell-matrix/cell-cell adhesion tension: all of which constitute a regulatory system, with efferent/afferent arms, that is likely responsive to IOP differential across the TM tissue. (25–27)

Various classes of adenosine agonists may also lower IOP by increasing trabecular outflow (28), with several receptor subtypes (A_1, A_{2A}, and A_3) in development as glaucoma therapeutics. Selective adenosine A_1 agonist INO-8875 (Inotek) is thought to increase trabecular outflow by reducing cell volume and remodeling the extracellular matrix following secretion of matrix metalloproteinases. (29) Novel adenosine A_{2a} receptor agonist OPA-6566 (Acucela and Otsuka Pharmaceuticals) is thought to lower IOP in human patients by stimulating aqueous humor outflow via the TM (30). A_{2A} receptors mediate vasodilatation, coupling through G proteins to stimulate adenylyl cyclase, and may be down-regulated after chronic exposure to an agonist (31, 32). A_3 / A_1 receptor agonist CF-101 (Can-Fite BioPharma) is an orally administered compound that showed IOP lowering efficacy in a phase II clinical trial aimed at reducing symptoms of dry eye (33). A_3 receptor agonists are thought to reduce IOP by inhibiting Cl^- channels of the nonpigmented ciliary epithelial (NPE) cells at the aqueous surface of the ciliary epithelium, reducing aqueous humor production (34–36).

Prostaglandin analogs (PGs) that target the EP_2 and EP_4 receptors may also increase outflow through the TM pathway. A selective prostanoid EP_4 receptor agonist (3,7-dithia PGE1) lowered IOP and increased total outflow facility in monkeys. No effect was seen on uveoscleral outflow or aqueous flow, suggesting that a substantial proportion of the ocular hypotensive activity was due to increased trabecular outflow facility. (37) Further studies with 3,7-dithiaPGE using human cell cultures and a whole-eye organ perfusion system showed that human SC and TM cells do express PG-EP_4 receptors and their activation in the human conventional pathway results in a significantly increased outflow facility.(38) The prostanoid EP_2 receptor agonist butaprost is thought to lower IOP by increasing uveoscleral outflow (39) but other EP_2 receptor agonists (e.g. Taprenepag isopropyl [formerly known as PF 04217329]) appear to be additive to latanoprost, (40, 41) suggesting that there may be a different mechanism of action with this class of compounds

Combination molecules

Most new TM drugs will want to demonstrate additivity and compatibility with the currently most prescribed compounds, the PGs, which lower IOP by increasing uveoscleral outflow. Additional daily drops become burdensome for patients and adherence can decrease. Combination formulations were developed that combine 2 mechanisms, 2 targets, 2 molecules into a single drop. Initial fixed-dose combinations all contained the β-adrenergic antagonist timolol but newer combinations include prostaglandins as well. A novel combination compound in development by Bausch and Lomb is Latanoprostene Bunod,

which combines 2 mechanisms and 2 targets in 1 molecule. Latanoprostene bunod (BOL-303259-X) 0.024% is a nitric oxide (NO)-donating prostaglandin F2α agonist that is rapidly metabolized in situ to latanoprost acid (to target uveoscleral outflow) and BDMN, a NO-donating moiety (to target TM outflow) (42). Aerie Pharmaceuticals has two fixed dose combination products in development. AR 13324 consists of an RKI (to target the TM) and a norepinephrine transporter inhibitor (to target aqueous humor inflow). Aerie's PG324 compound consists of 2 molecules, 3 mechanisms, 3 targets achieved by combining AR 13324 with PG latanoprost (to target uveoscleral outflow). If the B&L and Aerie products were combined the resulting compound would have 2 molecules, 4 mechanisms, 3.5 targets.

Drug delivery

Determining the best methods for getting the drugs to the target tissue, at an effective dose, while minimizing issues of patient adherence is a complex process. Topical drops are easy for patients to use but adherence wanes over time and with increasing numbers of drugs/doses. Preservatives, such as benzalkonium chloride, used in the more cost effective multi-dose bottles, can cause ocular surface issues (43, 44) and may contribute to worsening of chronic conditions such as glaucoma. (45 – 50)

Newer topical drop formulations aim to increase ocular bioavailability through manipulation of solution viscosity or corneal penetration by polymers, collagen shields, gels, nanoparticles, microemulsions and liposomes. (51, 52) Nanoparticles developed specifically for ophthalmic use are often polymeric colloidal particles in which the therapeutic agent is either encapsulated in a polymer (nanocapsule) or dispersed in the polymer matrix (nanosphere). (53) An advantage to these systems is that they can be engineered to be relatively cell-specific.

While injections and implants are commonly used to deliver drugs to both the anterior and posterior chambers, these can be somewhat invasive procedures and do entail some risk to the patient. A variety of novel drug delivery strategies are in development. Suprachoroidal injection using hollow microneedles may be a less invasive way to target the delivery of drugs to the choroid and retina than the commonly performed intravitreal injections. (54) Encapsulated cell technology implants have been used to deliver ciliary neurotrophic factor over a period of up to 2 years in patients with retinitis pigmentosa (RP) and geographic atrophy (GA). (55) Drug levels remained stable in the eye while CNTF, anti-CNTF antibodies, and antibodies to the encapsulated cells were not detected in the serum of patients, indicating no systemic exposure response. (55,56) Minimally invasive glaucoma surgical techniques might also be used to deliver drugs directly to the relevant tissues.

Gene transfer

A longer term drug delivery option in early phase clinical trials for retinal disease is gene therapy, where the goal is to reprogram target cells to up or down regulate a biochemical / physiological process to make more or less of something. Gene therapy strategies for glaucoma include increasing conventional outflow, increasing uveoscleral outflow, decreasing aqueous humor production and neuroprotection including rescue, regeneration & targeting of retinal ganglion cell (RGC) axons and RGC soma. (57)

Gene therapy has achieved some success in retinal applications where it has been used to improve vision in people with the retinal disease Leber's congenital amaurosis (58 – 61) and recently choroideremia (62), a rare type of inherited eye disease. These trials have reported positive results, which have helped further the field of ocular gene therapy by providing much needed safety and efficacy data for viral vectors (specifically adeno-associated virus or AAV vectors), paving the way for future therapeutics. The NEI has a unit on ocular gene therapy focused on developing AAV vectors for clinical disease targets of X-linked retinoschisis, retinitis pigmentosa, and macular degeneration. (63) In addition, the FDA is continuing to develop and refine guidance documents regarding cellular and gene therapy product development. These are necessary to clarify the special considerations for monitoring and follow-up include immunogenicity, persistence, migration, shedding, and growth and development. (64)

In animal models, experiments have demonstrated that AAV and scAAV (self-complementary adeno-associated virus) viral vectors can be safely delivered to the trabecular meshwork and that expression of a GFP reporter gene can be stably expressed and monitored serially and non-invasively for 2+ years. (65, 66) (FIG 2) Experiments delivering feline immunodeficiency virus (FIV)–based lentiviral vectors encoding elements of the prostaglandin pathway (COX-2, PGFSynthase, FP receptor) resulted in long term expression and decreased IOP lasting for the 5 month duration of the experiments in cats. The combination of the COX-2^{co} and FPR^{co} vectors produced the largest decrease in IOP. (67) Experiments injecting viral vectors encoding the cDNA for bovine PGF synthase in monkey eyes showed a significant decrease in IOP for 5 months. (68) Further work is needed to bring this promising technology to the clinic. Issues to be addresses include viral toxicity, regulation of gene expression - turning the gene on/off, immune/inflammatory responses and localization of transfection and/or gene activity. (69)

Gene therapy constructs for retinal applications are commonly delivered via subretinal injection. While not without risks, (70) the procedure delivers the vector directly to the affected area. In much the same way, gene therapy constructs for glaucoma could potentially be delivered directly to Schlemm’s canal via canaloplasty (71) or other minimally invasive surgical techniques. Benefits of this approach include the ability to use smaller volumes/ doses of vector and delivering it directly to an area where resistance to outflow is known to occur. (72) Regardless of the delivery method or location, it is likely that some cells other than the target cell type will be transduced by viral vectors. Whether that is detrimental or not remains to be determined.

Stem cells

As with gene therapy, retinal applications for stem cell therapies are in a more advanced stage of development than those for glaucoma. A recent review paper lists 20+ clinical trials using a variety of cell based therapies for retinal degenerative diseases including human embryonic stem cell (hES), bone marrow stem cell (BMSC), mesenchymal stem cell (MSC), human neural stem cell (hNSC) and induced pluripotent stem cells (iPSC). (73) Some of the positive aspects of gene transfer and stem cell applications are the same – there is a relatively small area/number of cells to replace, the eye offers visualization of the transplant

site allowing visualization of effects directly, serially and non-invasively, but there is a fundamental difference in approach. While gene therapy is focused on preserving the remaining cells in a degenerative disease environment (e.g. retinal ganglion cells in glaucoma or photoreceptor cells in macular degeneration), delaying the onset or progression of degeneration, stem cell therapy is intended to regenerate or replace lost tissue. Embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells and retinal stem cells have all been investigated for their potential in restoring cell types and the functionality lost in retinal diseases. (74)

Knowledge gained from the study of human embryonic stem cells and mammalian somatic cell reprogramming has led to the production of human induced pluripotent stem cells (hiPSCs). hiPSCs have potential for use in transplantation, high throughput drug screening, cell-culture disease modeling, disease gene discovery, and gene therapy testing, (75) though there are limitations on the number of cell passages that can occur before key cytological and functional attributes are lost. (76) One of the biggest allures of hiPSCs is the ability to derive patient-specific material for both clinical and research purposes. Allogeneic transplantation may avoid potential complications due to immune rejection. Based on efficacy studies in Royal College of Surgeons (RCS) rats, whose retinal dystrophy is characterized by RPE loss and secondary photoreceptor degradation, clinical trials for atrophic AMD and Stargardt macular dystrophy using subretinal injections of dissociated hESC-RPE have been initiated. Another hiPSC RPE cell trial that utilizes monolayer sheets of cells to treat exudative AMD has been announced. (77) Prefabricated RPE monolayers could also be used for combined transplantation of photoreceptors and RPE to treat diseases where both of these cell types are lost. (78, 79)

Stem cell based therapeutics for glaucoma present a formidable challenge. Replacement RGCs would need to extend axons down the optic tract to specific terminal connections in the lateral geniculate nucleus (LGN), functionally integrating into the complex circuitry of the inner retina and extending a lengthy axon capable of synapsing at precise brain targets. (74) Transplanted neural progenitors have been reported to invade the optic nerve and grow substantial distances; opening the prospect that over longer time periods transplanted RGC axons could reach their targets. (80) Highlighting the intense interest in this field the NEI Audacious Goals Initiative is to “Regenerate Neurons and Neural Connections in the Eye and Visual System”.

Intravitreal mesenchymal stem cell (MSC) transplantation can slow RGC death in a rat model of optic nerve damage. The neuroprotective effects are thought to be due to MSC secretion of factors from the platelet-derived growth factor family, indicating that the MSC implants could mediate retinal ganglion cell neuroprotection and that platelet-derived growth factor may be a target itself for realizing retinal ganglion cell neuroprotection. Autologous transplantation of MSC is a possibility since they can be isolated from bone marrow aspirates from individual patients and expanded in vitro before transplantation.(81) MSCs have also been programmed/engineered to secrete brain-derived neurotrophic factor (BDNF). When injected intravitreally in rats, they demonstrate some degree of RGC neuroprotection. (82 – 84)

A class of glial cell that has been studied for transplantation for neuroprotection is the olfactory ensheathing cell (OEC). OECs produce several neurotrophic factors that have been studied for glaucoma treatment, including BDNF, ciliary neurotrophic factor (CNTF), nerve growth factor (NGF), and glial-derived neurotrophic factor (GDNF). (85 – 87) In a rat model of optic nerve injury, OECs were transplanted into the optic nerve sheath and NTF was delivered intravitreally. This combination prolonged RGC survival and some functional benefit was noted (improved flash visual-evoked potential latency and amplitude) up to 8 weeks post-optic nerve crush. (88, 89) As noted above for MSCs, autogologus therapeutic transplantation may be possible with OECs, as they can be readily isolated from patient nasal mucosa biopsies.

Another stem cell strategy for glaucoma is to target the outflow pathway. Human TM stem cells (TMSCs) can be isolated, expanded in vitro and transplanted back into the anterior chamber where they home to the TM.(90) and exhibit phagocytic behavior. (91) After injection, TMSCs remained viable for 4 months and and reduced IOP in rats. (91)

To fully appreciate the effects of these novel treatment strategies, techniques to visualize and quantify changes in the relevant tissues/structures are needed and are being developed.

Imaging methods

Advances in imaging techniques have led to greater understanding of the disease process and how to treat it. Diagnostic imaging has added another element to the armamentarium for detecting and monitoring disease progression and the various instruments/techniques are becoming more useful to measure efficacy in clinical trials.

Structural variations that occur in glaucoma include changes in the optic nerve head, thinning of the retinal nerve fiber layer (RNFL) and ganglion cell-inner plexiform layer (GCIPL), and atrophy of the lateral geniculate nucleus (LGN) and visual cortex, which includes layer shrinkage and reduced neuron size and numbers. (92) The correlation between axon size and number and LGN atrophy has been demonstrated in experimental models of glaucoma. (93, 94) In humans, the thickness of the visual cortex, measured by magnetic resonance imaging (MRI), correlated positively with RNFL thickness, measured with a Topcon 3D OCT-1000. (95)

Neuroimaging using several types of MRI instruments has also shown LGN degeneration in human glaucoma. Using a 1.5-Tesla MRI, LGNs from glaucoma patients with bilateral visual-field defects and age-matched controls were identified and imaged. LGN heights were significantly decreased in glaucoma subjects compared to controls. (96) Using a 3.0-Tesla MRI, researchers found that LGN maximum height was negatively correlated with optic disk damage as assessed by cup-to-disc ratio. (97) An ultra-high field 7.0T MRI and a Cirrus sdOCT instrument were used to determine that LGN volumes in POAG patients were significantly smaller than those of age and gender-matched healthy controls. Furthermore, in patients, LGN volume was significantly correlated with GC-IPL thickness of the contralateral eye. (98) Together these studies indicate that LGN atrophy may have potential as a biomarker of visual system injury or glaucoma progression in some patients, especially those with media clarity issues. (99, 100)

A variety of imaging techniques were used to demonstrate the presence of lymphatic drainage channels in human, sheep and rodent eyes, including immunofluorescence with D2-40 antibodies for podoplanin, and LYVE-1 antibodies; Iodine-125 radio-labeled human serum albumin; and quantum dot tracers respectively. (101–103) It was recently determined that mice treated with latanoprost had increased lymphatic drainage from the eye by using hyperspectral imaging at multiple times following topical application of latanoprost and intracameral injection of quantum dots as a tracer. (105) This newly identified outflow pathway may be a new target for glaucoma therapeutics.

Early identification of cellular degeneration in glaucoma, perhaps even before irreversible vision loss occurs, and monitoring disease progression are key goals for several systems aimed at imaging apoptosis in RGCs. One system uses fluorescently labeled annexin V to non-invasively visualize single retinal cells undergoing apoptosis in vivo using a wide-angle confocal laser scanning ophthalmoscope (cLSO). This has been given the acronym DARC (Detection of Apoptosing Retinal Cells). (105) A range of in vivo studies using experimental models has been performed using DARC technology for the determination of RGC apoptosis. (106) (FIG 3) Modifications of the cSLO instrument enabled simultaneous detection of multiple, spectrally distinct markers. This allowed identification and quantification of nerve cells in the early and late phases of apoptosis and necrosis in different disease models: an Ab model of RGC death (intravitreal Ab25_35), recently shown to induce RGC apoptosis in rodent eyes (107); a model of staurosporine (SSP), induced neuronal apoptosis (108); an experimental glaucoma rat model of ocular hypertension (OHT) (109) and a triple transgenic Alzheimer's disease (AD) model (3xTg-AD) (110). The latter is a model of AD, which overexpresses APPSwe and tauP301L, as well as carries a PS1M146V knock-in mutation, and is currently the only existing transgenic model with both Ab and tau neuropathology. DARC will soon be tested in a glaucoma Phase I clinical trial (ISRCTN59484478) where the number of cells undergoing apoptosis at a given time point will be measured, with the goal of determining whether repeated imaging is useful as an indicator of disease progression.

A second system in development for serial, non-invasive imaging of apoptosis is TcapQ488, which uses a cell-penetrating caspase- activatable peptide probe. (111) Initial ex-vivo studies validated highly specific uptake by RGCs following intravitreal injection of fluorophores conjugated to a modified cell-penetrating peptide sequence and subsequent localization of apoptosing cells using retinal flat mounts from a rat model of NMDA-induced RGC degeneration. (112, 113) (FIG 4) In subsequent in-vivo studies using the same rat model and a confocal scanning laser ophthalmoscope (CSLO), probe activation was characterized. Sequential non-invasive fluorescence fundus imaging of individual animals showed that the time course of the probe activation signal reached near maximal at 12 hours and remained steady to 72 hours post injection. Electroretinogram (ERG) test showed no evidence of probe toxicity. An advantage of these cell penetrating peptides is that they can be modified to deliver other molecular imaging probes to RGCs or deliver probes with enhanced uptake by other retinal cell types. (114)

The results from both imaging systems demonstrate the potential of this type of technique, not only for direct assessment of retinal ganglion cell health in neurodegenerative diseases

such as glaucoma and Alzheimers, where increases and decreases in apoptotic activity can help guide treatment decisions and aiding the tracking of disease, but also to provide an assessment of the potential neuroprotective effects of novel drug candidates and their therapeutic efficacy. The development of a new and meaningful clinical endpoint would help fill an unmet need in glaucoma research and in the development of therapeutics. (115)

Conclusion

Advances in techniques that further our understanding of glaucoma pathophysiology (including continuous modeling of IOP) help inform development of novel therapeutics (including biodegradable implants) for glaucoma patients. Better drugs, better delivery methods and better patient evaluation and monitoring can enhance patient outcomes by giving physicians better tools to refine and personalize treatment strategies for this multifaceted disease.

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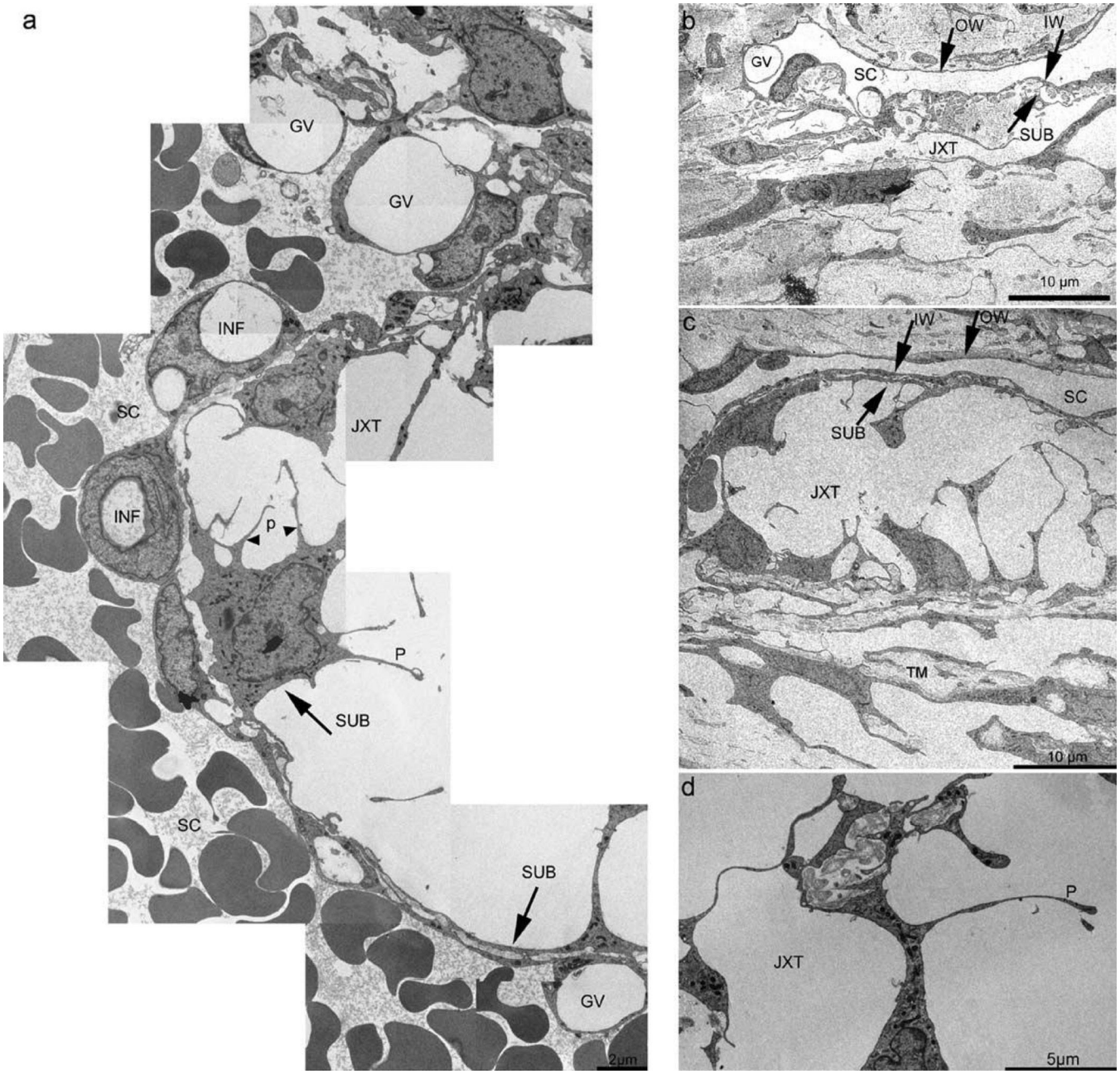

Figure 1.

Transmission EM of the trabecular meshwork (TM) following LAT-B (a, c and d [K554]) or vehicle (b [K596]). In (a), a long ‘montage’ of images is shown, depicting the IW - JXT regions of the TM following LAT-B. Panel (b) shows normal JXT region and its circumjacent structures; (c) indicates the massive ‘ballooning’ of the JXT region and the retention of close contact between IW and SUB (compare to (b)); (d) shows the absence of organelles from processes, irregular diameter of processes, and the entrapment of extracellular matrix deposits in intercellular spaces. GV, giant vacuoles; INF, membrane infoldings; IW, inner wall; JXT, juxtacanalicular region; OW, outer wall; P, cellular processes; SC, Schlemm’s canal; SUB, sub-canalicular cells. With permission from Sabanay

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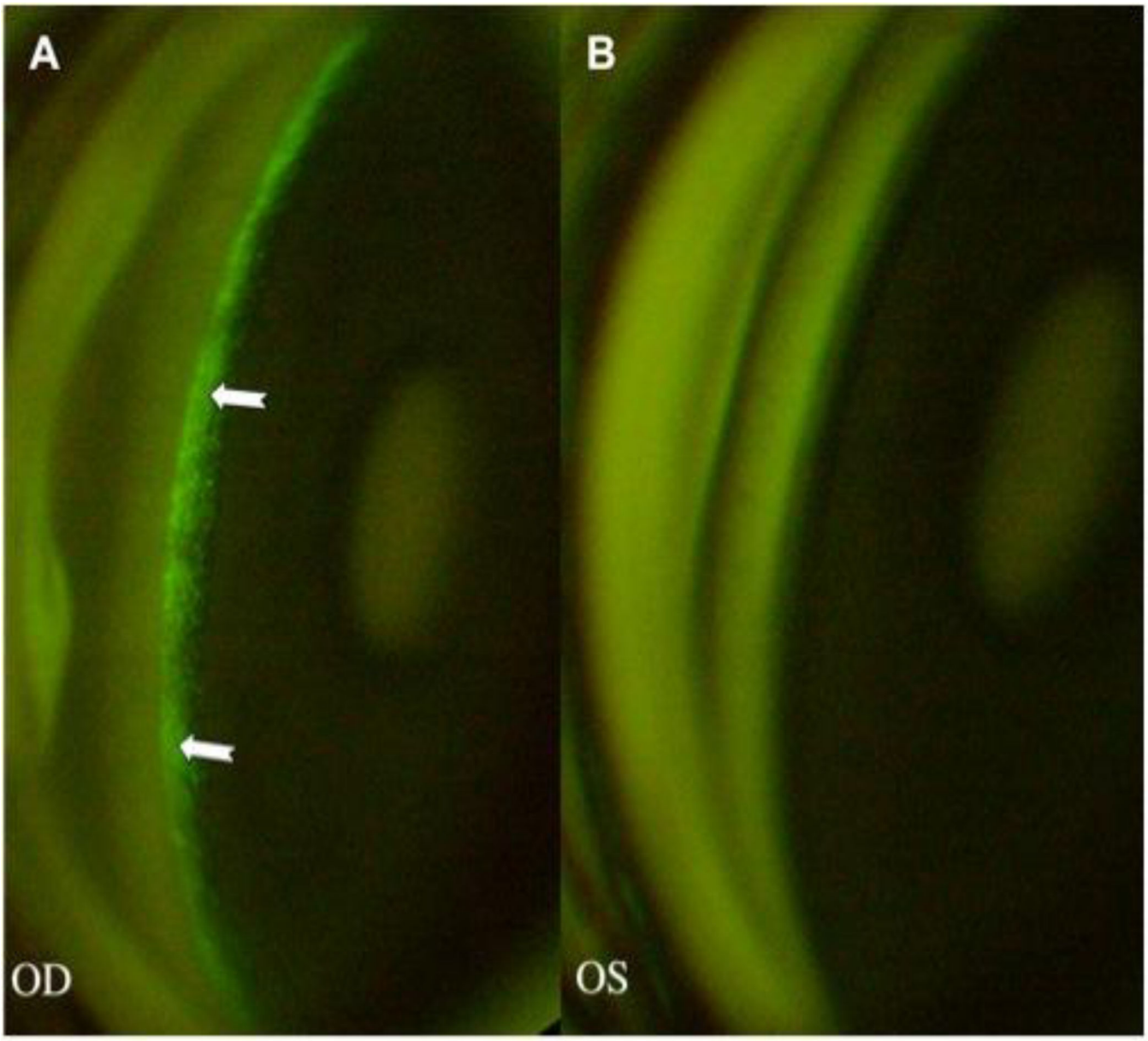

Figure 2.

Fluorescence, localized in the TM (arrows), is seen 5 days post-injection in an eye that received 1.0×10^8 TUs of an eGFP-expressing FIV vector. B: The control eye, injected with an equivalent volume of saline, shows no fluorescence. With permission from Liu X, Brandt CR, Rasmussen CA, Kaufman PL. Ocular drug delivery: molecules, cells, and genes. Can J Ophthalmol. 2007 Jun;42(3):447-54. Review.

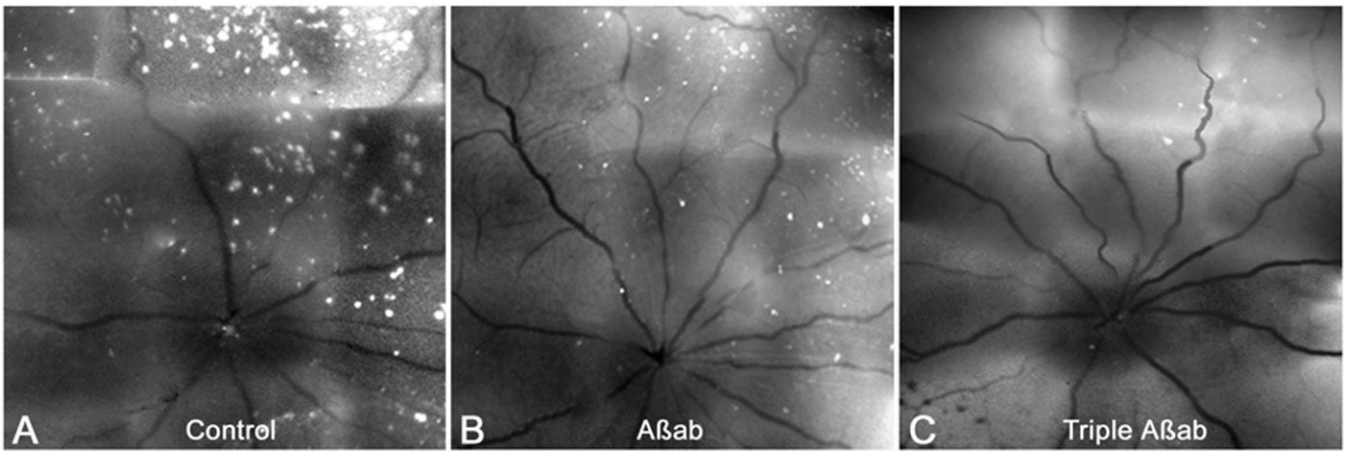

Figure 3.

Effects of combination Aβ-targeting therapy on RGC apoptosis. Compared to nontreatment control (A), DARC imaging shows triple therapy (C, Aβab+CR+βSI) was more effective than Aβab alone (B) in reduction of RGC apoptosis in an OHT model. With permission from Guo L1, Cordeiro MF Assessment of neuroprotection in the retina with DARC. Prog Brain Res. 2008;173:437-50. doi: 10.1016/S0079-6123(08)01130-8.

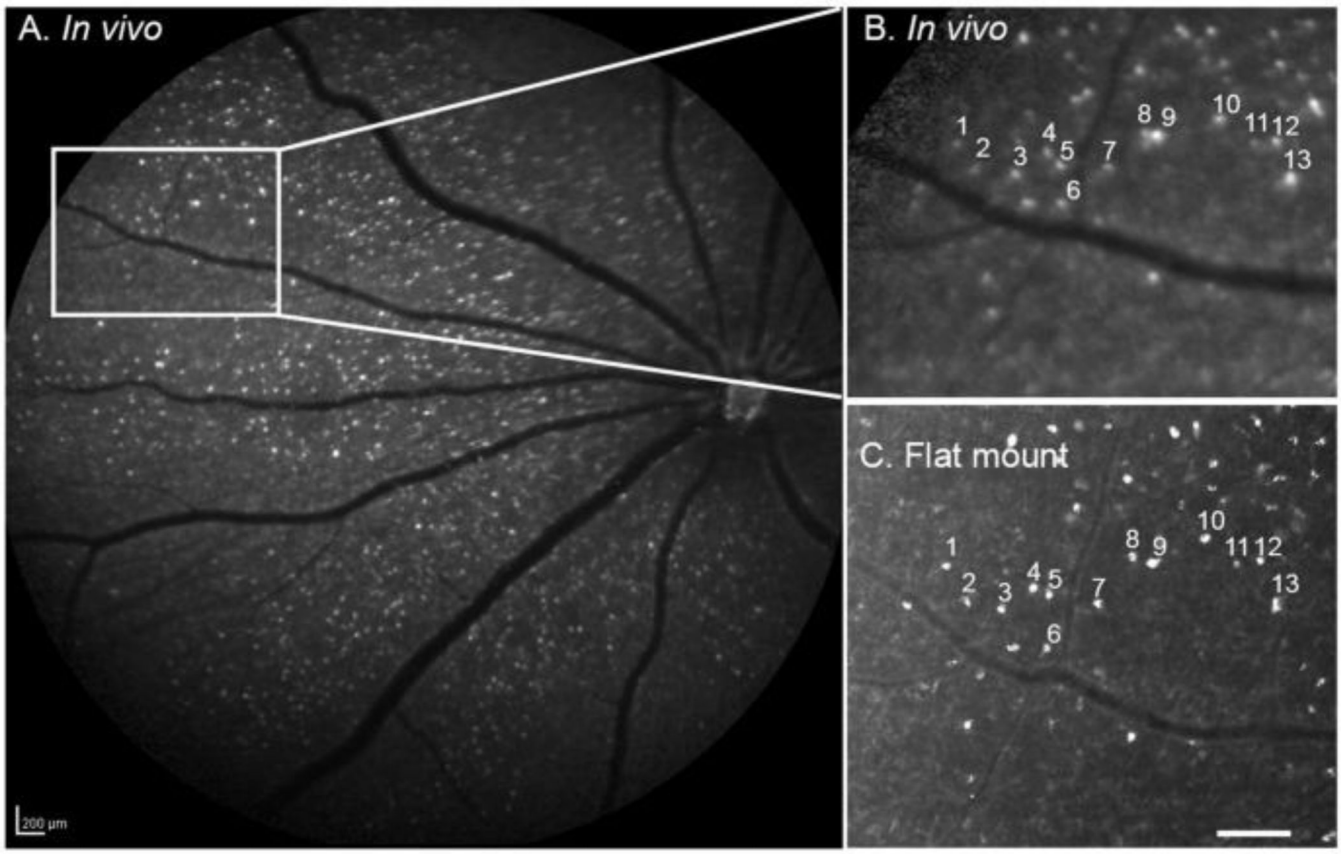

Figure 4.

(A) Fluorescent fundus image obtained in vivo using the CSLO (28 hours post-probe injection) from a rat eye pretreated with NMDA followed by TcapQ488. Strong, punctate fluorescent signals were detected in the retina ganglion cell (RGC) layer. (B) Higher magnification of the boxed area in A in which prominent fluorescent signals are highlighted. (C) Ex vivo flat mount of the same retina showed excellent correspondence with in vivo images in A and B, indicating that real time images reflect single cell resolution of probe activation. Scale bar: A, 200 µm; C, 100 µm. With permission from Qiu X, Johnson JR, Wilson BS, Gammon ST, Piwnica-Worms D, Barnett EM. Single-cell resolution imaging of retinal ganglion cell apoptosis in vivo using a cell-penetrating caspase-activatable peptide probe. PLoS One. 2014 Feb 21;9(2): :e88855. doi: 10.1371/journal.pone.0088855. eCollection 2014.