Highlights of ARVO 2007

A selection of the papers and posters presented this year at the annual meeting in Fort Lauderdale, Florida.

BY NEERU GUPTA, MD, PHD

t the 79th meeting of the Association for Research in Vision and Ophthalmology, experts from around the world presented their newest findings. Only a few of the hundreds of novel ideas and discoveries in glaucoma are highlighted here.

IMAGING SINGLE RETINAL GANGLION CELLS LIVE

Using an adaptive-optics scanning laser ophthalmoscope, researchers successfully imaged single ganglion cells in monkeys' retinas in vivo. They imaged the cell bodies, major dendrites, and axons of individual cells at high contrast and resolution. These cells were labeled with rhodamine dextran retrogradely transported from the lateral geniculate nucleus.¹

LONGITUDINAL IMAGING OF THE DEGENERATION OF RETINAL GANGLION CELLS

Investigators demonstrated that the progressive degeneration of retinal ganglion cells can be studied in vivo. Thy-1 expression is down-regulated prior to these cells' death, and the researchers used this change as an indicator of the cells' functional integrity. Optic nerve crush was performed in transgenic mice engineered with fluorescently labeled Thy-1 retinal ganglion cells. The investigators found a consistent and progressive loss of fluorescent profiles after injury to the optic nerve.²

GENE THERAPY LOWERS IOP

Gene transfer produced significant and sustained decreases in IOP in cats for up to 5 months. Because topical PGF2 α reduces IOP, investigators hypothesized that stable prostaglandin biosynthesis could be engineered in vivo that would have therapeutic value. They took advantage of cyclooxygenase-2, a rate-limiting enzyme

in prostaglandin biosynthesis. Coupled with a PGF2 α receptor transgene, ectopic cyclooxygenase-2 expression produced reductions in IOP that were sustained and comparable to existing pharmaceuticals. Fluorophotometry established increased outflow facility rather than decreased aqueous production as the mechanism.³

IMAGING THE RETINAL GANGLION LAYER

The intravitreal injection of colchicine into monkeys' eyes disrupted microtubules within the axons of retinal ganglion cells. The microtubules' disruption was detected as a decreased thickness of the retinal nerve fiber layer by scanning laser polarimetry but not by optical coherence tomography (OCT) in nonhuman primates. The different physical properties detected by these two imaging technologies may be complementary and provide additional information on axonal degeneration.⁴

RAPID COUNTS OF RETINAL GANGLION CELLS

Researchers described a novel, automated, high-throughput method for counting retinal ganglion cells using a modified scanning system and the Brn-3a—immunolabeled, whole, flat-mounted retinas of rats. This type of approach may represent an important technological advance in high-throughput drug-screening studies requiring the processing of tissue and the analysis of cellular viability.⁵

IMAGING IN GLAUCOMA PATIENTS

Using anterior segment OCT, researchers performed a dynamic analysis of dark-light changes of the anterior chamber angle in subjects with normal and narrow angles. Although the angle's width generally decreased linearly with increasing pupillary size, differences in the width measured in the dark versus the light varied substantially among individuals. This finding suggests the

importance of standardizing lighting conditions for the evaluation of the anterior chamber angle.⁶

Investigators used high-speed, swept-source, three-dimensional OCT as a novel method to analyze the anterior segment noninvasively after glaucoma surgery. The technique may be useful for structural assessments, including after trabeculectomy or laser iridotomy.⁷

OXIDATIVE INJURY IN HUMAN GLAUCOMA

The volume of advanced glycation end products seems to be higher in the anterior chamber angles of patients with glaucoma. Investigators compared trabeculectomy specimens from individuals with primary open-angle glaucoma and enucleation specimens from patients with secondary glaucoma against age-matched controls. The immunoreactivity of advanced glycation end products was more evident in glaucomatous specimens and was most obvious in secondary glaucoma. This study suggests that oxidative-type changes contribute to the pathology of the anterior chamber angle in glaucoma.⁸

Researchers assessed oxidative activity in the aqueous humor by measuring lipid peroxidation products, malondialdehyde, and antioxidant activity (total reactive antioxidant; superoxide dismutase and glutathione peroxidase). The markers of oxidative stress were significantly higher in the aqueous humor of 208 patients with primary openangle glaucoma compared with 284 controls prior to cataract surgery. Antioxidant therapies may be relevant to these findings.⁹

THE CONJUNCTIVA IN GLAUCOMA

Researchers compared the mast cell profiles in the conjunctivae of glaucoma patients undergoing filtration surgery with those of a nonglaucoma control group. The number of mast cells appeared to be higher in medicated glaucoma patients, glaucoma patients who had previously undergone surgery, and uveitic glaucoma patients. The difference was significant in patients undergoing repeat trabeculectomy. These results suggest that mast cell activity may contribute to the postoperative wound healing process and the increased risk of excessive conjunctival scarring after glaucoma filtration surgery.¹⁰

A HIGH-PRESSURE GLAUCOMA MODEL

Investigators developed a glaucoma model in which they injected 15-µm latex beads into the anterior chambers of Brown Norway rats. The researchers observed no ocular inflammatory responses. They successfully increased IOP by 30% with a single injection and by 30% to 40% with two injections for 6 weeks. This model allows for further assessment of the shrinkage and loss of retinal ganglion cells' axons in glaucoma.¹¹

IOP AND THE NOCTURNAL PERIOD

IOP seems to increase during the nocturnal period. Outflow facility measured by tonography did not decrease sufficiently during the nocturnal period to compensate for the lessened rate of aqueous humor flow. Other possible factors include changes in episcleral venous pressure and/or uveoscleral flow rate.¹²

VENOUS PULSATIONS AND INJURY TO THE OPTIC NERVE HEAD

Spontaneous retinal venous pulsation, present in most normal subjects, is absent in many glaucoma patients. It can be induced by applying graded pressure to the eye as ophthalmodynamometric force. Researchers measured the predictive value of ophthalmodynamometric force and IOP in determining glaucomatous progression and found them to be strongly predictive of and independently associated with the optic disc's excavation. The measurement of venous pulsations may be relevant to clinical practice.¹³

PSEUDOEXFOLIATION GLAUCOMA

In the Reykjavik Eye Study, investigators detected definite pseudoexfoliation (PXF) in either eye of 108 of the subjects (10.7%) they examined. The prevalence increased significantly with subjects' age, and females were more frequently affected than males (12.3% vs 8.7%). PXF was associated with higher IOP compared to eyes without slit-lamp evidence of PXF. The study found no significant association between PXF and corneal thickness, lenticular opacification, or optic disc size. 14

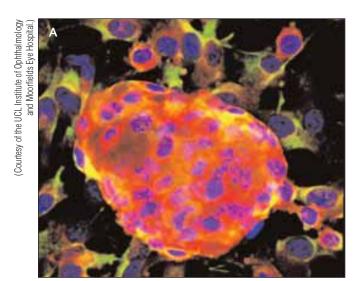
The prevalence of PXF in the Thessaloniki Eye Study also increased with subjects' age. Excluding patients with glaucoma showed that the presence of PXF material was not associated with higher IOP or differences in cup-to-disc ratios compared to subjects without PXF.¹⁵

THE PLACEMENT OF SURGICAL SHUNTS

Researchers prospectively studied corneal endothelial density in eyes implanted with aqueous shunts. They assessed the relationship between the shunt's position within the anterior chamber (using anterior segment OCT) and changes in the density of corneal endothelial cells in glaucoma patients. The results suggest that the entry position close to the peripheral cornea may have an important influence on the corneal endothelium's long-term function.¹⁶

NEUROPROTECTION

Memantine, an open-channel N-methyl-D-aspartic acid receptor blocker, blocked N-methyl-D-aspartic acid-induced increases in the spiking activity of retinal



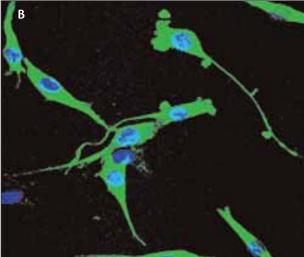


Figure 1. Researchers isolated neurospheres formed by Müller stem cells from the human neural retina. The cells express the Müller cell marker cellular retinaldehyde binding protein (red) and the neural stem cell marker nestin (green). Yellow staining indicates coexpression of both markers (A). Human Müller stem cells can be induced to differentiate in vitro into cells that express HuD (green), a protein predominantly expressed by ganglion and amacrine cells (B). Blue staining in A and B indicates cells' nuclei.

ganglion cells at a concentration that had little or no effect on retinal light signaling observed under controlled conditions in the retinas of rabbits. Memantine is currently under investigation as a neuroprotective agent in the treatment of glaucoma.¹⁷

HUMAN RETINAL DEGENERATION

Researchers quantified retinal changes at all levels in human glaucoma using multiphoton confocal microscopy combined with DAPI staining. The measurement of these changes in retinal flat mounts stained with DAPI confirmed the selective loss of retinal ganglion cells in human glaucoma. It also pointed to a gradient of transneuronal degeneration that was greater in the inner than outer nuclear layer. These findings suggest that neuroprotection in glaucoma should be designed to target all of the retinal layers affected by degeneration.¹⁸

PROGENITOR CELLS TO RETINAL GANGLION CELLS

A study of adult human Müller-like progenitor cells MIO-M1 showed that it was possible to induce them to differentiate into cells expressing retinal ganglion cell phenotype in vitro. By altering the extracellular environment, these Müller-like cells expressed transcription factors associated with the development of retinal ganglion cells (Figure 1). They therefore may be a useful source of progenitors to form retinal ganglion cells in vitro.¹⁹

TRANSPLANTATION

The intraocular transplantation of the neural stem cells MIO-M1 in a rat glaucoma model eliminated the transplanted progenitor cells within days. Even in immune-suppressed animals, researchers noted macrophage infiltration and a reduction in graft survival. This study helps to explain the critical role of the local ocular environment, including the immune system, in the survival and integration of progenitors within a diseased retina.²⁰

NANOTECHNOLOGY

This year's ARVO Annual Meeting included an exciting nanotechnology workshop, moderated by Robert Ritch, MD, and Marco Zarbin, MD, PhD. The session highlighted basic applications of nanotechnology to vision-related research and the restoration of sight. This rapidly evolving field is highly relevant to the treatment of ocular disease and spans regenerative medicine, tissue bioengineering, drug delivery, microsensors for IOP, and photo-switches for restoring sight—to name only a few of the many potential applications for glaucoma.

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Li Ka Shing Knowledge Institute of St. Michael's Hospital; and Director, Institute of Medical Sciences Summer Research Program, University of Toronto. She is a consultant to Alcon

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RESEARCH RESULTS

Laboratories, Inc.; Allergan, Inc.; Merck & Co., Inc.; and Pfizer Inc. Dr. Gupta may be reached at (416) 864-5444; guptan@smh.toronto.on.ca.

1. Williams DR. In vivo imaging of monkey retinal ganglion cells. Paper presented at: The 2007 ARVO Annual Meeting; May 8, 2007; Fort Lauderdale, FL.

2. Leung CK, Lindsey JD, Liu Q, et al. Longitudinal in vivo imaging of retinal ganglion cells after optic nerve crush in a transgenic mice model. Paper presented at: The 2007 ARVO Annual Meeting; May 8, 2007; Fort Lauderdale, FL.

2007; Fort Lauderdale, FL.
3. Barraza RA, McLaren J, Poeschla EM. COX-2 pathway gene therapy for glaucoma: sustained IOP reduction with integrated prostaglandin biosynthesis and response transgenes. Paper presented at: The 2007 ARVO Annual Meeting; May 8, 2007; Fort

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put, retinal ganglion cell counting system. Poster presented at: The 2007 ARVO Annual Meeting; May 9, 2007; Fort Lauderdale, FL. 6. Cheung C, Leung CKS, Dorairaj S, et al. Dynamic analysis of dark-light changes of the anterior chamber angle with anterior seg-ment OCT. Paper presented at: The 2007 ARVO Annual Meeting; May 7, 2007; Fort Lauderdale, FL.

7. Kawana K, Oshika T, Miura M, et al. Clinical application of highspeed swept-source three dimensional optical coherence tomography for analyzing anterior eye segments after glaucoma surgery. Paper presented at: The 2007 ARVO Annual Meeting; May 8, 2007; Fort Lauderdale, FL.

 Pillunat LE, Zubaty V, Spoerl E, et al. Advanced glycation end-products (AGEs) in the anterior chamber angle of patients with glau-coma. Paper presented at: The 2007 ARVO Annual Meeting; May 7, 2007; Fort Lauderdale, FL.

9. Gallego-Pinazo R, Pinazo-Duran MD, Vinuesa Silva I, et al. Oxidative stress in the aqueous humor of primary open angle glau-coma patients. Poster presented at: The 2007 ARVO Annual Meeting; May 10, 2007; Fort Lauderdale, FL.

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cell profile in the conjunctival of glaucoma patients. Poster presented at: The 2007 ARVO Annual Meeting; May 9, 2007; Fort Lauderdale, FL.

11. Sappington RM, Calkins DJ. Shrinkage and loss of RGC axons in a new latex bead rat model of high-pressure glaucoma. Paper presented at: The 2007 ARVO Annual Meeting; May 8, 2007; Fort

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12. Sit AJ, Nau CB, McLaren JW, et al. Circadian variation of aqueous dynamics in adults 18-45 years old. Paper presented at: The 2007 ARVO Annual Meeting; May 7, 2007; Fort Lauderdale, FL.

13. Morgan WH, Balaratrasingam C, Hazelton ML, et al. Venous interest and proceedings of the proceedings of

pulsation characteristics are predictive of increased optic disk excavation independently of intraocular pressure. Paper presented at: The 2007 ARVO Annual Meeting; May 8, 2007; Fort Lauderdale, FL. 14. Arnarsson AM, Damji KF, Sverrisson T, et al. Prevalence of pseudoexfoliation and association with IOP, corneal thickness, and structural optic disc parameters in the Reykjavik Eye Study. Paper presented at: The 2007 ARVO Annual Meeting; May 7, 2007; Fort auderdale, FL

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16. Hau SC, Scott A, Bunce C, Barton K. Corneal endothelial morphology in eyes implanted with aqueous shunts. Poster presented at: The 2007 ARVO Annual Meeting; May 9, 2007; Fort Lauderdale,

17. Hare WA. Block of NMDA-induced activity in retinal ganglion cells by memantine. Poster presented at: The 2007 ARVO Annual Meeting; May 9, 2007; Fort Lauderdale, FL.

18. Lei Y, Garrahan N, Johnson DH, et al. Quantification of retina

transneural changes in human glaucoma using a novel multiphoton-DAPI method. Paper presented at: The 2007 ARVO Annual Meeting; May 7, 2007; Fort Lauderdale, FL.

19. Singhal S, Bhatia B, Khaw PT, Limb GA. Potential of MIO-M1 cells to differentiate into retinal ganglion cells (RGC) in vitro. Poster presented at: The 2007 ARVO Annual Meeting; May 9, 2007; Fort

Lauderdale, FL. 20. Bull ND. Limb GA. Martin KB. Intraocular transplantation of adult human Müller progenitor cells (MIO-M1) in a rat glaucoma model. Poster presented at: The 2007 ARVO Annual Meeting; May 9, 2007; Fort Lauderdale, FL.