

A MOUSE MODEL OF ELEVATED INTRAOCULAR PRESSURE: RETINA AND OPTIC NERVE FINDINGS

BY **Ronald L. Gross MD**,* Jianzhong Ji MD PhD, Peter Chang MD, Mark E. Pennesi PhD, Zhuo Yang BS, Jian Zhang PhD, AND Samuel M. Wu PhD

ABSTRACT

Purpose: To develop and characterize a mouse model of elevated intraocular pressure (IOP) as a means to investigate the underlying cellular and genetic mechanisms of glaucomatous optic neuropathy.

Methods: An experimental increase in IOP was induced in one eye of each adult C57BL/6J mouse by argon laser photocoagulation of the episcleral and limbal veins. The IOP of both eyes of each mouse was measured using an indentation tonometer prior to treatment and once a week thereafter. The mouse retinal ganglion cells (RGCs) were identified immunocytochemically using an antiserum against Thy1,2, CD90.2, and the number of RGCs was measured with confocal microscopy. The reduction in the number of RGCs was compared in the experimental and control eyes. The mechanism of RGC death after IOP elevation was investigated using TdT-mediated dUTP nick end labeling (TUNEL) staining. The pathologic changes of optic nerve following elevated IOP were characterized by light and electron microscopy.

Results: After laser treatment, mean IOP was increased in the treated eyes from the control mean of 13 ± 1.8 mm Hg to 20.0 ± 2.8 mm Hg at 4 weeks. Peak IOP was 32 ± 2.5 mm Hg in the experimental group. RGC loss was $16.9\% \pm 7.8\%$ at 2 weeks ($n = 6$, $P < .05$) and $22.4\% \pm 7.5\%$ at 4 weeks ($n = 6$, $P < .05$) after laser photocoagulation. TUNEL staining showed that there were marked increases in the number of apoptotic nuclei in the ganglion cell layer in the treated eyes; moreover, these TUNEL-positive cells were mostly distributed in the peripheral areas of the retina. The optic nerve axons from the eyes with elevated IOP were observed to demonstrate greater degeneration compared with the control group.

Conclusions: The magnitude and duration of the elevation of the IOP supports the use of this model as a surrogate for glaucomatous optic neuropathy. The presumed apoptotic mechanism of RGC death is consistent with this assumption. Laser-induced increased IOP appears to be a viable means for future investigations of the genetic mechanisms of glaucoma

Trans Am Ophthalmol Soc 2003;101:163-172

INTRODUCTION

Primary open-angle glaucoma is the most common form of glaucoma and the second leading cause of blindness. It is defined by characteristic anatomic damage of the optic nerve, including optic disc cupping and loss of retinal ganglion cells (RGCs) and the resulting functional defects of the visual field. The pathophysiology and the optimal treatment of glaucoma are still under investigation. It is widely accepted that the level of intraocular pressure

(IOP) is a consistent risk factor in its incidence, severity, and progression and that reducing IOP is the only currently available treatment for glaucoma that has been shown to decrease the risk of progressive glaucomatous optic nerve damage. However, it is also accepted that IOP is not the only risk factor and that other parameters, such as optic nerve perfusion and the intrinsic susceptibility of the RGCs, may well play a key role in many if not most patients with glaucoma.

Investigations directed toward greater understanding of the mechanisms and damage from glaucoma have made use of animal models of elevated IOP. These have included the monkey, rat, and rabbit models. However, for many reasons, the mouse is a more desirable model to use, since it provides an anatomic structure similar to that in humans and there is extensive experience and capability of genetic manipulation in the mouse. Because the globe of the mouse is small, standard techniques to

From the Department of Ophthalmology, Cullen Eye Institute, Baylor College of Medicine, Houston, Tex. Supported by an unrestricted grant from Research to Prevent Blindness; grants EY04446 and NIH Vision Core EY02520 from the National Institutes of Health; the Retina Research Foundation of Houston; and the Neva and Wesley West Foundation.

*Presenter.

Bold type indicates **AOS** member.

noninvasively measure IOP using available tonometers are not possible, and thus previous efforts to raise IOP in mouse eyes could not be evaluated. Invasive tonometers were developed to measure the IOP in mice;¹ however, repetitive measurements were not feasible. Recently, noninvasive devices have been developed, including a modified Goldmann applanation tonometer² and a plunger device;³ also, we have developed an indentation-type tonometer to reliably measure mouse IOP (Chang P et al, unpublished results). Now that IOP can be measured, it is possible to attempt to develop and characterize a mouse glaucoma model without associated developmental anomalies.

Because the level of IOP is a major risk factor in glaucoma, experimental glaucoma models have been developed to increase IOP primarily by using different methods to block the outflow of aqueous humor. Gaasterland and Kupfer⁴ described experimental IOP elevation in monkeys using excessive direct application of argon laser to the trabecular meshwork. Although this is anatomically the closest model to human glaucoma, this technique is limited by the expense involved and the high and variable pressures obtained. Later, models of increased IOP in rats were developed using various techniques to block aqueous egress from the eye. These included hyperosmotic saline microinjection into limbal veins, cauterization of the large veins draining the anterior segment, and ablation of the limbal vessels with a laser to decrease outflow.⁵⁻⁹ In all of these models, standard tonometers are used to measure IOP.¹⁰ However, currently, specific investigations to explore the mechanisms of glaucoma damage and treatment using genetic manipulation are not possible with monkey or rat models. Thus, there exists the importance of developing a mouse model that will provide this potential capability.

For example, recent studies have suggested that damage to the RGCs in glaucoma is associated with apoptosis. The initial study of experimental glaucoma in monkeys indicated that dying cells exhibited many of the features known to be consistent with apoptosis. More rigorous morphological studies have recently confirmed these earlier observations. Further evidence of apoptosis in experimental glaucoma has been obtained by assaying ganglion cells for typical DNA fragmentation. More recent studies have extended and confirmed the TUNEL findings by demonstrating multiple markers of apoptotic degradation in RGC-layer cells in human retina from patients with glaucoma.¹¹ Thus, apoptosis is suggested to be the possible mode of RGC death in glaucoma. Therefore, a mouse model of elevated IOP would provide a unique and powerful tool to explore the mechanism of loss of RGCs in the experimental eyes. It appears that the death of RGCs is due to apoptosis, and if so, further inves-

tigations using strains of mice with known modifications of the apoptotic pathway could greatly expand our understanding of how RGCs die as a result of elevated IOP in glaucoma.

However, the first step is to develop and characterize a mouse model of elevated IOP in order to begin these studies. A genetically modified mouse strain, DBA/2J, has been developed and characterized.¹² It manifests anterior segment anomalies that presumably result in elevated IOP. However, the impact of these developmental changes on the mechanism of glaucoma damage is not known. Therefore, the development of a mouse model of elevated IOP in wild-type strains more likely will provide better insight into the underlying mechanisms and potential treatments for human glaucoma.

MATERIALS AND METHODS

All experiments were conducted and all laboratory animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Baylor College of Medicine Animal and Use Committee. Animals were housed under a 12-hour light/12-hour dark cycle with standard chow and water.

IOP MEASUREMENT IN A CHRONIC GLAUCOMA MODEL

Noninvasive IOP Measurement in Live Mice

A modified Schiotz-like indentation tonometer (Figure 1) was designed and manufactured using aluminum for the platform, stainless steel for the base, and Teflon-coated titanium for the plunger (Carbtext Corp, Houston, Tex). The plunger weighed 253.1 mg and had a tip diameter of 809 μm and contact area of 0.514 μm^2 . The tonometer was mounted on a micromanipulator to enable vertical and horizontal excursions.

Pressure Calibration

An enucleated, mounted globe from adult C57BL/6J mouse was cannulated with a sterile, 28-gauge needle and connected to a pressure transducer (model 90602; SpaceLabs Inc, Redmond, Wash) with a signal amplifier. Balanced salt solution (BSS) hanging on an intravenous pole was used to vary the IOP and maintain it at a given pressure by altering the height of the pole. This closed system was filled with BSS prior to cannulation to eliminate all air bubbles.

IOP Measurement

The tonometer was slowly lowered onto the mounted globe just until the first contact with the corneal surface was made. The position of the tonometer at the first contact was noted using the scale of the micromanipulator (Huxley-type,



FIGURE 1

Indentation tonometer measuring the intraocular pressure in a live mouse

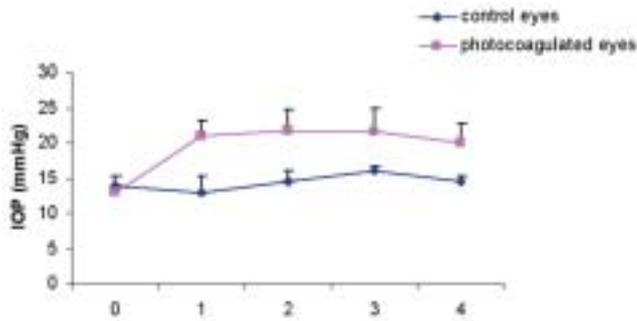


FIGURE 2

Comparison of intraocular pressure (IOP) between experimental and control eyes. In experimental eyes, the IOP was increased to 1.5 times above that of control eyes. Each data point and error bar represents the mean \pm SD IOP at each time point of measurement.

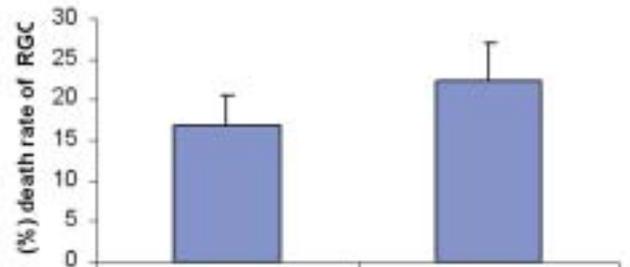


FIGURE 3

Graph showing retinal ganglion cell (RGC) loss 2 and 4 weeks after elevation of intraocular pressure. The number of RGCs was compared with that of the control group, and the difference was statistically significant ($P < .05$) for both 2 weeks and 4 weeks.

Westcoast Instruments, Palo Alto, CA). The tonometer was then further lowered just until the upward movement of the tonometer tip was noted. The position of the tonometer at the first upward movement of the tonometer tip was measured. By subtracting the two readings on the micromanipulator scale, the total excursion of the tonometer was calculated. The entire procedure was performed under direct visualization using a vertically mounted microscope.

Noninvasive IOP Measurement in Live Mice

After the mice were anesthetized using intraperitoneal

injection of ketamine (50 mg/kg) and xylazine (5 mg/kg), they were placed on a stainless steel platform and gently immobilized using head and tail holders. Body temperature was maintained using a gentle heating lamp. BSS was placed on the eye to prevent corneal dehydration and to facilitate visualization. The tonometer tip was lowered onto the cornea and the excursion of the tonometer was measured using the method described above. All the procedures were performed under direct visualization using a vertically mounted microscope. The mean of five consecutive measurements was used. IOPs were meas-

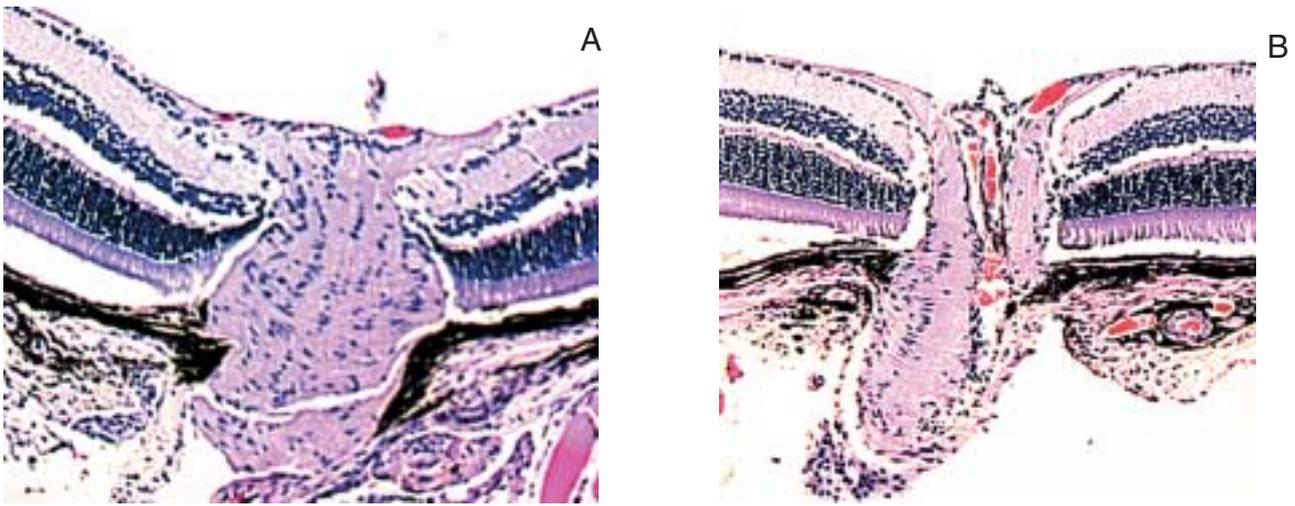


FIGURE 4

Light microscopic histopathology of the optic nerve showed expanded scleral canal and swelling in the region of the lamina cribrosa in the eye with elevated intraocular pressure with disruption of the nerve bundles and increased cellularity within the nerve head (A). There was no evidence of such changes for the control optic nerve (B).

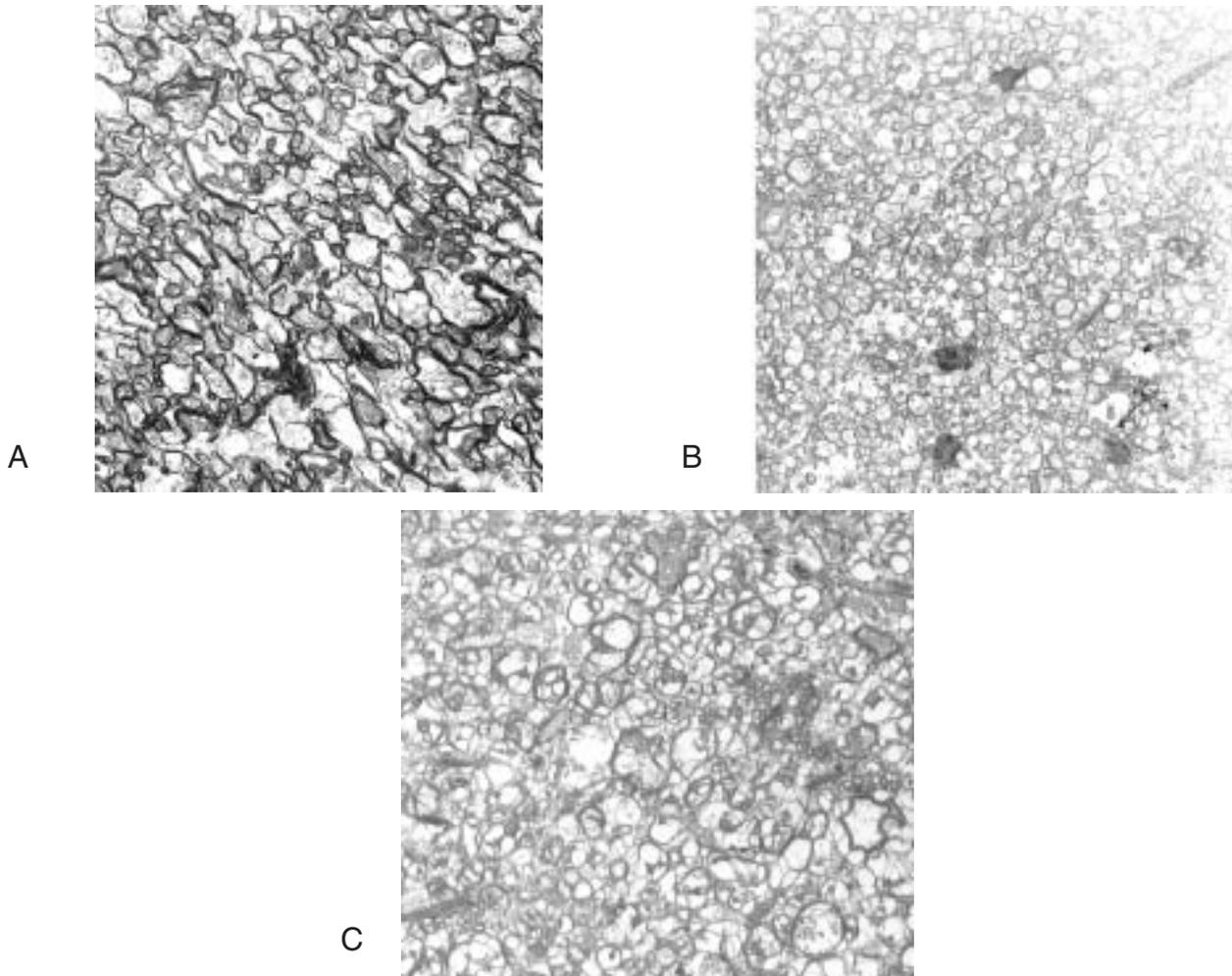


FIGURE 5

Electron microscopy of the transversely sectioned axons of the optic nerve from control, 2-week, and 4-week groups. A, Optic nerve from control group. B, Optic nerve from experimental group for 2 weeks survival. Axons were characterized by swelling, enlargement, and accumulation of organelles, vesicles, and dense bodies. There were also reactive macrophages in the treated optic nerve. C, Optic nerve from 4-week treated group. There were more serious degenerative changes compared with 2-week group.

ured prior to laser treatment and at least weekly thereafter.

ELEVATION OF IOP IN MICE

Experimental elevation of IOP was induced unilaterally in 12 C57BL/6J mice less than 5 weeks old. Animals were anesthetized with intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5mg/kg) and placed on warming pads throughout the procedure. One eye of each animal underwent argon laser photocoagulation of the episcleral and limbal veins. A spot size of 50 μm , duration of 0.1 second, and power of 80 to 110 mW were applied to the visible vasculature within 1 mm of the limbus over 360° using green-only argon laser (Coherent Inc, Santa Clara, Calif). The corneas were moistened with BSS as needed throughout the procedure.

PATHOLOGIC EVALUATION

Enucleated eyes were processed and paraffin sections were stained by hematoxylin-eosin. The anterior chamber angle, retina, optic nerve, and vessels were observed using light microscopy. Electron microscopic evaluations of the anterior 2-mm segments of the optic nerves of eyes were obtained from untreated mice and at 2 and 4 weeks following elevated IOP. The specimens were placed in electron microscopy fixative overnight at 4°C. After washing in 0.1M phosphate buffer, tissue samples were post-fixed in 1% phosphate-buffered osmium tetroxide, then dehydrated in ethanol and embedded in Epon. During embedding, the nerves were carefully oriented so that complete transverse section across the nerve trunk can be cut. Semithin (0.5- μm) transverse sections were obtained from each of the blocks using a Reichert-Jung ultramicrotome with glass knives made on a LKB knife-maker. These sections were stained with toluidine blue. Ultrathin sections with silver interference color were also obtained, stained with uranyl acetate, and examined with a JEOL 100SX electron microscope.

GANGLION CELL IMMUNOLABELING

Mice were killed by overdose of ketamine-xylazine anesthesia and the eyes were removed. Retinas were extracted and whole-mounted on slides and then were stained by Thy1,2, CD90.2, a specific ganglion cell surface marker. The nucleus of each cell was stained by Top3. Confocal microscopy of retinal flat-mounts was used to count the positive cells and take photographs. The number of RGCs was measured at intervals along the median line of each quadrant (6 to 7 microscopic fields per quadrant) from the optic disc to the peripheral border of the retinas. A total of 24 to 28 microscopic fields were counted per retina, corresponding to approximately 3% to 3.2% of the retinal area. The average density of RGCs was calculated for the

entire retina. To account for the loss of RGCs at different survival times in each treated group, changes in the densities of RGCs were expressed as percent loss of RGCs comparing the laser-treated and contralateral control eyes from the same animals.

TUNEL STAINING FOR RETINAL SECTIONS

Retinas prepared for TdT-mediated dUTP nick end labeling (TUNEL) staining and analysis were divided into three groups: (1) non-laser-treated eyes, which served as controls ($n = 3$); (2) and (3) laser-treated animals, which were sacrificed at 2 weeks ($n = 3$) and 4 weeks ($n = 3$) after laser. Both eyes of all animals were enucleated and fixed in 4% paraformaldehyde for 1 hour. Flat-mounted retinas were cut transversely along the superior-inferior axis of the globe to ensure comparability. Retinal sections were prestained by Thy1,2 and washed three times in phosphate buffered saline (PBS) (pH 7.4). The sections were permeabilized in 0.1% Triton X-100 and 0.1% sodium citrate solution for 5 minutes followed by blocking in 3% H_2O_2 for 15 minutes. Sections were incubated in TUNEL reaction mixture solution (enzyme solution and label solution; Roche, Germany) overnight. Slides were rinsed three times in PBS for 5 minutes each. Sections were then evaluated under the confocal microscope.

STATISTICAL ANALYSIS

Paired two-tailed t test was used for evaluation of study results.

RESULTS

INTRAOCULAR PRESSURE

The mean IOP in animals under general anesthesia and before treatment was 13 ± 1.8 mm Hg. All treated eyes in this study had elevated IOP by the third day after laser photocoagulation (Figure 2). The mean IOP in the laser-treated eyes was 20 ± 2.8 mm Hg, statistically significantly higher than that in the control eyes at each time point during the first 4 weeks following laser treatment ($P < .01$). There was no need to repeat laser treatment after the first session, since an adequate elevation of IOP was obtained and maintained over the study period.

Loss of RGCs After Laser Photocoagulation

In the first 2 weeks following laser photocoagulation, the percentage of RGCs lost in the treated eyes with elevated IOP compared to the untreated controls was $16.9\% \pm 7.8\%$. This was a statistically significant difference ($P < .05$). At 4 weeks, the death rate of RGCs was $22.4\% \pm 7.5\%$, which was also a statistically significant difference between control and glaucomatous groups ($P < .05$) (Figure 3).

TUNEL Staining

Positive TUNEL staining cells were observed only in the ganglion cell layer. These retinas were prelabeled by Thy-1,2, and therefore the positive cells were also found to be stained red from Thy1, 2 staining. This confirms that the positive cells are only RGCs. Positive TUNEL cell staining was detected in very few cells (3 ± 1) in each section from control retinas. At 2 weeks postlaser, the number of TUNEL-positive ganglion cells in each section was increased significantly (25 ± 3 , $P < .01$). At 4 weeks after laser, there were still TUNEL-positive RGCs detected (18 ± 2 , $P < .01$).

Optic Nerve Changes

Light microscopic histopathology of the optic nerve demonstrated an expanded scleral canal in the region of the lamina cribrosa in the eye with elevated IOP. There was swelling and disruption of the nerve bundles with increased cellularity within the nerve head (Figure 4). There was no evidence of synechia formation in the anterior chamber angle. Electron microscopy showed the transverse sectioned axons of the optic nerve from a mouse with elevated IOP at 4 weeks to be abnormally enlarged and swollen. The axons were characterized by accumulation of organelles, vesicles, and dense bodies indicative of blocked axonal transportation. Moreover, there were also reactive macrophages in the treated optic nerve. There were no such changes in the axons of the optic nerve from the control eyes (Figure 5).

DISCUSSION

The mouse has been extensively studied in many experimental fields because of the feasibility of using transgenic technology, as well as the relative ease of handling and relatively low cost when compared with other mammals.¹² Moreover, the mouse eye has many structural similarities to the human eye, including a well-defined trabecular meshwork, Schlemm's canal, ciliary body, and vascularized retina.¹³ However, due to the small size of the mouse eye and technical difficulty in measuring IOP, a chronic model of elevated IOP without associated anatomical anomalies to study glaucoma has never been established in mice. The development of noninvasive tonometry in mice has opened the possibility of developing and characterizing a mouse model of elevated IOP. The noninvasive tonometer we have developed and used for this study demonstrates an adequate level of accuracy for this purpose. A mouse strain with elevated IOP has been described; however, in this model there are associated developmental anterior segment anatomic abnormalities, making assessment difficult and potentially affecting the response to IOP elevation.¹²

The glaucoma model of elevated IOP in rats has been successfully established and characterized using methods similar to those used in this study. The laser photocoagulation was used to decrease aqueous outflow and increase the IOP. This mouse model successfully produced elevated IOP in nearly 90% of treated eyes, with a mean pressure of 23 mm Hg and a highest IOP of nearly 45 mm Hg. Two and 4 weeks after treatment, the number of RGCs had been reduced by 16% and 23%, respectively, compared to control, untreated eyes, and some RGC loss was present in most eyes. The damage was found to be confined to the RGC layer (using Thy1,2 labeling) and optic nerve axons. In the rat model of elevated IOP induced by argon laser, it was shown that there was a twofold IOP increase over baseline, resulting in a 27% to 30% loss of RGCs at 2 months. In this mouse model, a 1.5-fold increase in IOP in experimental eyes resulted in a 23% loss of RGCs at 4 weeks. The similarity of these models is that there is a modest elevation of IOP and chronic RGC death in experimental eyes. However, there is a slight difference in death rate and level of IOP between these two models. For this mouse model, the power, duration, and number of laser applications are less than those in the rat because of the smaller scale of the mouse globe. However, our data suggests that this mouse model represents a valid model of elevated IOP with loss of RGCs.

There is evidence to suggest that ganglion cells die in spontaneous or experimental glaucoma by apoptosis. Much of what we know at the basic science level has been from the study of experimental glaucoma in monkeys. In this primate model, early light and electron microscope observations of the RGCs indicated that dying cells exhibited many of the features we now know to be consistent with apoptosis.¹⁴ Further evidence of apoptosis in experimental glaucoma has been obtained by assaying ganglion cells for DNA fragmentation. Monkeys exhibit at least 10 times more TUNEL-positive RGCs in eyes with experimental glaucoma than in fellow control eyes.¹⁵ More recent studies on rats with elevated IOP have also confirmed the TUNEL findings supporting apoptotic RGC death. Our study showed that TUNEL-positive cells were present exclusively in the ganglion cell layer in the experimental eyes, and few positive cells were found in control eyes. Interestingly, more positive cells were observed at the peripheral areas of the retina compared with the central areas. Our study confirmed that apoptosis is responsible for RGC loss in this mouse glaucoma model.

There is another factor suggested to contribute to RGC death after elevation of IOP. Axonal degeneration is presumed to be induced by the deprivation of trophic factors to the RGC body, thus signaling the RGCs to undergo apoptosis and die. It has been demonstrated that

transport of axonal material is blocked at the scleral lamina cribrosa by IOP elevation.¹⁶ The loss of anterior nerve fibers, combined with posterior and lateral movement of the lamina cribrosa, leads to an increase in optic disk cupping.¹⁷ In our study, we found there was swelling of the optic nerve and an enlarged optic disc in the experimental eye compared with the control eye. These findings are similar to those in the developmental glaucoma mouse model.¹² This result suggests that the problem of axonal transport may also be involved in the death of RGCs in this model.

The ultimate purpose of the development of a mouse model of elevated IOP is to use it to better understand and hopefully be more effective in treating and preventing human glaucoma. Recently, several different chromosomal loci have been identified that are associated with primary open-angle glaucoma and also congenital and juvenile glaucoma, aniridia, Axenfeld-Rieger syndrome, as well as other secondary glaucomas. These candidate genes will open new horizons of investigation. The use of a mouse model has the potential to greatly facilitate these inquiries.

In conclusion, our study provides evidence for RGC death in mice with elevated IOP. Moreover, the loss of RGCs appears to be due to apoptosis and is associated with axonal degeneration. Given these findings, it makes this mouse model a viable tool in the investigations of glaucoma aimed at slowing the progression of the neurodegenerative process.¹⁸ Given the numerous candidate genes identified in human glaucoma, there is no doubt that genetic and molecular advances made possible by this model represent powerful techniques in the ongoing investigations as to the mechanisms of glaucoma and gene function in the future.

REFERENCES

1. Avila MY, Carre DA, Stone RA, et al. Reliable measurement of mouse intraocular pressure by a servo-null micropipette system. *Invest Ophthalmol Vis Sci* 2001;42:1841-1846.
2. Cohan BE, Bohr DF. Measurement of intraocular pressure in awake mice. *Invest Ophthalmol Vis Sci* 2001;42:2560-2562.
3. Danias J, Kontiola, Filippopoulos T, et al. Method for the noninvasive measurement of intraocular pressure in mice. *Invest Ophthalmol Vis Sci* 2003;44:1138-1141.
4. Gaasterland D, Kupfer C. Experimental glaucoma in the rhesus monkey. *Invest Ophthalmol* 1974;13:455-457.
5. Shareef SR, Garcia-Valenzuela E, Salierno A, et al. Chronic ocular hypertension following episcleral venous occlusion in rats. *Exp Eye Res* 1995;61:379-382.
6. Sawada A, Neufeld AH. Confirmation of the rat model of chronic, moderately elevated intraocular pressure. *Exp Eye Res* 1999;69:525-531.

7. Mittag TW, Danias J, Pohorenc G, et al. Retinal damage after 3 to 4 months of elevated intraocular pressure in a rat glaucoma model. *Invest Ophthalmol Vis Sci* 2000;41:3451-3459.
8. Woldemussie E, Ruiz G, Wijono M, et al. Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. *Invest Ophthalmol Vis Sci* 2001;42:2849-2855.
9. Hare W, Ton H, Woldemussie E, et al. Electrophysiological and histological measures of retinal injury in chronic ocular hypertensive monkeys. *Eur J Ophthalmol* 1999;(9 Suppl 1):S30-S33.
10. Moore CG, Milne ST, Morrison JC. Noninvasive measurement of rat intraocular pressure with the Tono-Pen. *Invest Ophthalmol Vis Sci* 1993;34:363-369.
11. Wax MB, Tezel G, Edwards PD. Clinical and ocular histopathological findings in a patient with normal-pressure glaucoma. *Arch Ophthalmol* 1998;116:993-1001.
12. John SW, Anderson MG, Smith RS. Mouse genetics: a tool to help unlock the mechanisms of glaucoma. *J Glaucoma* 1999;8:400-412.
13. Duke-Elder S, Dobree JH. The formation of avascular connective-tissue bands in proliferative diabetic retinopathy. *Bibl Ophthalmol* 1968;76:133-138.
14. Quigley HA. Ganglion cell death in glaucoma: pathology recapitulates ontogeny. *Aust N Z J Ophthalmol* 1995;23:85-91.
15. Quigley HA, Nickells RW, Kerrigan LA, et al. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. *Invest Ophthalmol Vis Sci* 1995;36:774-786.
16. Neufeld AH, Hernandez MR, Gonzalez M. Nitric oxide synthase in the human glaucomatous optic nerve head. *Arch Ophthalmol* 1997;115:497-503.
17. Quigley HA, Miller NR, George T. Clinical evaluation of nerve fiber layer atrophy as an indicator of glaucomatous optic nerve damage. *Arch Ophthalmol* 1980;98:1564-1571.
18. Lichter PR. Genetic clues to glaucoma's secrets. The L. Edward Jackson Memorial Lecture, Part 2. *Am J Ophthalmol* 1994;117:706-727.

DISCUSSION

DR PAUL R. LICHTER. The paper by Gross and colleagues is important in showing that increased IOP in the mouse eye induced by argon laser photocoagulation of episcleral and limbal vessels can cause retinal ganglion cell death by apoptosis. As the authors pointed out, because the mouse can be genetically manipulated quite effectively, this animal provides outstanding opportunities to study glaucoma mechanisms for disease causation and prevention.

However, to effectively use mouse models to study the effects of IOP on retinal ganglion cell death and ways to prevent it, what is needed first is an in vivo IOP measuring system that can be relied upon to be accurate and reproducible within a very small range of variability. Only in this way will it be possible to determine what subtle

changes in IOP may mean to ganglion cell health. I commend the authors on their beautifully conducted study to show how increased IOP can destroy mouse retinal ganglion cells by apoptosis. My main questions about the study relate to the tonometer.

The authors sent me a graph of the mouse IOP indentation tonometer readings plotted against the true transducer readings where IOP is controlled by cannulation of an enucleated mouse eye. Variability from a linear relationship yields an R2 value of 0.8753. On first thought, this seems like a very good R2 value. In some situations it might be. However, in this case, the authors' tonometer is trying to replace the perfectly accurate readings of the transducer and, therefore, must have very high accuracy. An R2 value more in the range of 0.95 or higher would be desirable if we are to trust that the tonometer might be an adequate replacement for the transducer. To illustrate this point, pretend that the same graph is showing the relationship of blood pressure with age. An R2 of 0.8753 would indicate a strong correlation. But it wouldn't mean that knowing the age of the patient could substitute for taking his or her blood pressure. By the same token, this R2 is not high enough to mean that we can use the authors' tonometer readings as a substitute for the transducer readings.

By contrast, the recent report of an induction-impact tonometer scaled for the size of the mouse eye appears to show promising results in terms of its ease of use and its accuracy.¹ Compared to the device reported today by Gross and colleagues, the induction-impact tonometer may provide more reliable readings. A similar graph on the induction-impact tonometer shows an R2 value of 0.959.

Imagine, for a moment, that we wanted to validate a new tonometer to use on our patients. We would insist on intra- and inter-observer accuracy of the instrument to within 1-2 mm Hg. To be useful in the kinds of genetic animal studies on glaucoma to which Gross and colleagues allude, even greater accuracy and repeatability will be needed in measuring mouse IOP. The indentation tonometer presented today was calibrated on a cannulated enucleated mouse globe wherein IOP was varied under controlled conditions. Since the authors knew what the actual IOP was in this enucleated eye, did they have readings performed with their instrument in a masked manner where one person operated the instrument and another read the micromanipulator scale to determine its excursion? And did multiple readers measure the IOP multiple times to determine intra- and inter-reader variability under these IOP-controlled conditions? Were power calculations determined to know how many readings and how many observers would be needed to validate the instrument's accuracy and reproducibility in the living

mouse? Did the authors consider using additional enucleated and cannulated globes with identical IOPs to determine whether their instrument obtained the same readings on different globes having the same IOP? Were the in vivo, non-invasive IOP readings on the twelve study animals obtained in a masked fashion to reduce bias as much as possible? These might be considered rhetorical questions since the authors are likely aware of the shortcomings of their instrument and their early attempts at its validation. I'd be interested to know, though, what the authors plan to do in future studies and whether they plan to evaluate the induction-impact tonometer since the validity of that instrument seems better than their own.

In summary, this paper is important in that it produces a mouse model of induced IOP elevation, develops an instrument to measure it, and looks at the histopathologic process in retinal ganglion cells as they are affected by elevated IOP. If the authors are able to validate their tonometer, they have the likelihood of making a lasting contribution to the field.

My thanks to Dr Gross for allowing me to see the preliminary manuscript well in advance of the meeting, for providing me with a manuscript under review that details the instrument studies, and for sending me his slides. I also wish to thank my colleagues, Drs. Philip Gage, Brenda Gillespie, Brett Hughes, and Julia Richards, for their advice in assessing this work.

REFERENCE

1. Danias J, Kontiola AI, Filippopoulos T, et al. Method for the noninvasive measurement of intraocular pressure in mice. *Invest Ophthalmol Vis Sci* 2003;44:1138-1141.

DR GEORGE L. SPAETH. Can you titrate the glaucoma and how long does it last? Can you make the pressure go up to what you think is 25 and then keep it 25 all day. And then make it go up to 35 the next week, and then 45... And how long can you keep the pressure at a particular level?

DR JOHN C. HECKENLIVELY. I've been working on the mouse model now since 1984, mainly to generate retinal degenerations and to work toward therapy through that model. As part of that project we have found a number of glaucoma models and have made them available at the Jackson Lab. One of the models we found was the DBA/2J model, and that is actually not a congenital glaucoma at all. There is no deformity of the angle. I've looked at the histology of that through the natural history, and basically they develop iris atrophy with loss of pigment into the trabecular meshwork. That occurs between about 4 and 6 months, and then the pressure goes up in those animals and they get ganglion cell loss. It's actually an excellent

model for pigmentary glaucoma. We have other models that may be of interest to the glaucoma specialists, both congenital and normotensive models as well as regular open-angle glaucoma.

My main concern is the validation of your tonometer. The standard way of validating the tonometer is to put a needle into the eye and to measure the pressure with a manometer. The mouse is an extremely powerful model, both genetically and in terms of the ocular structure since the mouse eye mimics the human eye in an amazing way. It has a bigger lens and doesn't have a macula. Otherwise the structures histologically look identical. The homology between human and mouse is quite high in terms of the genes at over 95%. So anything you find in the mouse is likely to be true in human.

There was another paper on a mouse tonometer at ARVO this year by Dr Tony Adamis' group. Did you need to use a micromanipulator with anesthesia? The problem with anesthesia is that can alter the intraocular pressure of the mouse. Most of examinations in our mice are performed as though they are little patients so that we hold them by the ruff of the neck and look in their eye and manipulate them. If you treat them gently it's very easy to do and you get realistic measurements that way.

DR E. MICHAEL VAN BUSKIRK. I hope you will not get distracted by the accuracy of your tonometer. I think our general field of glaucoma is becoming ominously distracted away from the optic nerve, toward obsessing about the measurement of the intraocular pressure. I too am interested in animal models of glaucoma my whole career, and although I am somewhat skeptical about the analogy to the human eye based on my own castings of the anterior segment years ago, the most exciting aspect of your model in terms of analogy to the human glaucoma, is that the pressure is only mildly elevated. I've often regretted that the tonometer just doesn't measure low, medium, and high. Most glaucomas are on the low side of medium and it appears to me that that's where yours is. I think it's much more applicable to the human glaucoma than most of the models that exist.

DR DAVID L. KNOX. I've had a chance to look at some experimental mouse eyes after investigators have tried to create an infarction of the nerve head. There's no cribiform plate in the mouse eye, which is a major anatomic variance from the human. In your study you're not measuring the blood pressure of the animal and you're thinking about 20 millimeters of mercury in a human eye, which is going to be very different from 20 millimeters of mercury in a mouse eye, where you don't know the blood pressure.

DR RONALD L. GROSS. To reply to Dr Lichter: Yes, for the

validation we did mask the person actually doing the measurement and the reader recognizing that was necessary. We did check interobserver and intraobserver variability. Power calculations were somewhat difficult because they have to be based on some assumption and unfortunately at the time these studies were performed, there was no available data to base it on. The graph that Dr Lichter showed was the comparison of the tonometer measurements that we obtained to cannulation of the anterior chamber with a needle attached to a transducer device. We did validate it that way. But, I'm not sure that we measure intraocular pressure all that accurately in humans with all the devices that we have. Given the r-squared value, we felt it was accurate enough to proceed toward our main intent to see if there was any chance at all that raising the pressure in mice resulted in something similar to human glaucomas. Calibration was done in multiple enucleated eyes.

We're actually in the middle of the duration study right now. We're out to about 12-16 weeks in some of these eyes. It appears that the pressure stays at this same level.

With additional ablation of these vessels would we get the pressure to go even higher? In the rat model you typically treat and then re-treat a week or so later to get and maintain that pressure elevation. We have not done that with these eyes and have not found the need.

About the DBA2J mouse, there is something different about the anterior segment of that mouse, with the pigment dispersion. Our concern was that if it involves the iris pigment epithelium, could it involve other structures as well that could change the response to elevated IOP? We didn't think that was well enough characterized at this point. Our preference was to go to a wild-type mouse if possible.

There are several other tonometers that have been recently reported. We started our studies prior to their availability. It was our intent under the circumstances to be able to get the next step, which is to be able to develop and characterize the model.

We agree with Dr Buskirk about avoiding distractions with the intraocular pressure. The strength of this model is that we are able to explore specifically what is going on in elevated intraocular pressure as far as damage to the retinal ganglion cells. I do not know if there is a correlation between the blood pressure and intraocular pressure, whether it is similar or not to humans.

