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Comparison of Anterior Segment Structures in Two Rat Glaucoma Models: An Ultrasound Biomicroscopic Study

Nikolaos Nissirios¹, Raul Chanis¹, Elaine Johnson², John Morrison², William O. Cepurna², Lijun Jia², Thomas Mittag^{1,3}, and John Danias^{1,4}

¹ Department of Ophthalmology, Mount Sinai School of Medicine, New York, New York

² Department of Ophthalmology, Oregon Health and Science University, Portland, Oregon

³ Department of Pharmacology, Mount Sinai School of Medicine, New York, New York

⁴ Department of Neuroscience, Mount Sinai School of Medicine, New York, New York

Abstract

Purpose—Optic nerve disease in chronic IOP elevation rat glaucoma models develops at different rates. This study was undertaken to investigate whether anterior chamber (AC) changes develop in two popular models in vivo and whether the changes are related to IOP.

Methods—Ten female Wistar rats and 12 male Brown-Norway rats were subjected to episcleral vein cauterization (EVC) and hypertonic saline episcleral vein sclerosis (HSEVS), respectively. Contralateral untreated eyes served as controls. IOP was recorded for a period of 5 to 6 weeks, and with the rats under anesthesia, the eyes were imaged with an ultrasound biomicroscope. Measurements of the AC depth (ACD), trabecular–iris angle (TIA), iris thickness at the thickest point near the pupillary margin (IT), angle opening distance (AOD; at 200 μ m from the scleral spur), and ciliary body area (CBA) were compared between control eyes of the two strains and between experimental and control eyes within each strain. The differences were correlated with IOP history.

Results—Eyes subjected to EVC demonstrated greater increases in IOP than eyes subjected to HSEVS. Between rat strains, control eyes differed significantly in all the parameters studied, except for ACD. No difference was detected between experimental and control eyes in the EVC group. In contrast, experimental eyes in the HSEVS group had ~71% larger ACDs and ~32% smaller CBAs than did the contralateral control eyes ($P < 0.001$). ACD and CBA correlated well ($R^2 = 0.80$ and 0.51 , respectively) with IOP in the HSEVS group. Two of the experimental eyes in this group showed the presence of ultrasound-scattering material in the AC.

Conclusions—Despite apparently higher IOP exposure, eyes in the EVC rat model of glaucoma do not undergo changes in the AC. In contrast, eyes subjected to HSEVS display deepening of the AC and reduction in size of the ciliary body within 5 to 6 weeks. These changes correlate to IOP exposure and may be the result of specific changes induced by the experimental intervention. These models are likely to rely on different mechanisms of pressure elevation and cannot be used interchangeably.

Rat models of glaucoma that involve chronic elevation of IOP are often used to study the retinal and optic nerve pathophysiology of the disease¹ as well as for screening potential neuroprotective agents.² These models presumably rely on occlusion of the outflow pathways,

Corresponding author: John Danias, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1183, New York, NY 10029; john.danias@mssm.edu.

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either at the level of the trabecular meshwork (TM)^{3,4} or at the level of the episcleral veins⁵ or both.⁶ It has often thus been assumed that models are similar and/or interchangeable. Although the effects of IOP elevation that ensues on the retina and optic nerve have been extensively studied, the effects on anterior chamber (AC) structures have not been investigated quantitatively.

Ultrasound biomicroscopy (UBM) has been successfully used in the study of anterior segment structures of humans with pigmentary glaucoma.^{7–10} There have also been reports of the use of UBM for the imaging of the anterior segment in mice with glaucoma associated with pigment release from the iris¹¹ as well as in normal rats.¹² In this study, we used UBM to investigate changes occurring in AC structures in two popular rat glaucoma models, induced by either episcleral vein cauterization (EVC)⁵ or hypertonic solution episcleral vein sclerosis (HSEVS),⁶ in an effort to determine whether they cause IOP elevation via similar mechanisms.

METHODS

Animals and IOP Elevation

All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

For EVC, female Wistar rats 180 to 200 g ($n = 10$) were used. With the rats under general (xylazine/acepromazine/ketamine) anesthesia, EVC was performed with a hand-held cautery, as previously described,¹³ on one eye of each animal.

For HSEVS, male Brown Norway rats (retired breeders, 8 months old; $n = 12$) were used. Hypertonic sodium chloride solution was injected in the episcleral veins of one eye of each animal under general anesthesia, as previously described.⁶ Both groups of animals were maintained for a period of 5 to 6 weeks after the experimental treatment before imaging. During this time, IOP was measured with a handheld tonometer (Tonopen; Medtronic, Jacksonville, FL) on at least a weekly basis. Brown Norway rats are docile animals and after a period of training, IOP was measured in the nonsedated state under topical anesthesia only. The Wistar rats required the administration of light sedation with a mixture of ketamine and xylazine to measure IOP. To avoid the effects of the anesthetics, IOP measurements were obtained within 2 minutes after the animal lost deep pain sensation. Animals in the EVC group were maintained in a 12:12 light–dark cycle, whereas Brown Norway rats were maintained for at least 5 weeks in a constant light cycle. All animals received food and water ad libitum.

IOP exposure of each eye in the two groups of animals was calculated by multiplying the area under the curve in an IOP-versus-time graph. IOPs between two readings were interpolated.

UBM Imaging of the Rat Anterior Segment

Rat eyes were imaged using a UBM with a 50-MHz transducer (Paradigm Medical Industries, Inc., Salt Lake City, UT). To allow for immersion biomicroscopic imaging, a custom-made eye cup was used.¹²

Under general (xylazine/acepromazine/ketamine) and topical (pro-paracaine 0.5%) anesthesia, a lateral canthotomy was performed to avoid pressure on the eye during imaging. The custom eye cup was placed over the exposed eye and filled with normal saline as a coupling solution. All eyes were imaged at the temporal angle.

Image Analysis

Measurements of the corneal thickness (CT), anterior chamber depth (ACD), trabecular-iris angle (TIA), angle opening distance (AOD), and iris thickness at the thickest point near the pupillary margin (IT) were obtained from collected images, as previously described,¹⁴ with the following modification. Because of the smaller size of the rat eye, the AOD was measured at 200 μm from the scleral spur. In addition, the size of the ciliary body was measured.¹² All images analyzed were from the temporal half of the eyes, as previously described. Measurements were performed in a masked fashion in triplicate for each image. Three images were analyzed for each eye.

Statistical Methods

The means $\pm$ SD were calculated for the means of all measured dimensions in each image. Control eyes in both groups were compared by *t*-test. Experimental eyes within each group were compared to control eyes by paired *t*-test. Cumulative IOP exposure, maximum IOP, and mean IOP (the average of all IOPs obtained during follow-up) was correlated with ACD and ciliary body size within the hypertonic saline sclerosis group.

RESULTS

IOP differences (ΔIOP) between the two eyes of animals in both the EVC and HSEVS group are shown in Figure 1. For maximum IOP, mean IOP, and cumulative IOP exposure the difference between experimental and control eyes in the EVC group was significantly higher than the respective values for the HSEVS group ($P < 0.01$, 0.00001, 0.00001 respectively, *t*-tests).

Values (mean $\pm$ SD) of all parameters measured in the control eyes of the two strains are shown in Table 1. The two strains had significantly different ACs for all parameters studied (*P* by *t*-test), except for the ACD. Brown Norway rats had narrower angles (lower TIAs), smaller AODs, thicker irides, and smaller ciliary bodies.

In comparison with control eyes, experimental eyes for the EVC group (Table 2) had no significantly different values for ACD TIA, AOD, IT, and ciliary bodies (paired *t*-test). A typical UBM image from both eyes of a rat in the EVC group is shown in Figure 2.

In contrast, for the HSEVS group, experimental eyes had significantly deeper ACs ($P < 0.000001$) and smaller ciliary bodies ($P < 0.0002$). TIA, IT, and AOD were not significantly different between the experimental and control eyes (paired *t*-test; Table 3). In some of the animals the deepening of the AC was rather dramatic (Fig. 3).

Maximum IOP, mean IOP, and cumulative IOP exposure correlated well with ACD in the HSEVS group ($R^2 = 0.79$, 0.80, and 0.79, respectively). They also correlated fairly well with the size of the ciliary body in the same animals ($R^2 = 0.51$, 0.47, and 0.41, respectively). The correlations of ACD and ciliary body size with mean IOP are shown in Figures 4 and 5, respectively.

Two of the 12 experimental eyes in the HSEVS group had echogenic material in the AC, consistent with inflammatory cells and debris (Fig. 6). None of the eyes in the EVC groups or in any of the control groups showed any such echogenic material in the AC.

DISCUSSION

Rats with chronically elevated IOP as a result of experimental intervention are popular models for studying glaucomatous optic nerve damage. To date, at least four different means of

increasing IOP have been devised. Two widely used methods involve occlusion of the episcleral veins by cauterization,⁵ and sclerosis of the episcleral veins and outflow pathways with hypertonic saline.⁶ Originally, the models involved different rat strains, but according to the literature they produced almost similar results in terms of IOP elevation.¹⁵ The EVC method has been used mainly in young Wistar rats,^{5,13} but it has also been used in older animals and in one study, Brown Norway rats.¹⁶ In contrast, the hypertonic saline injection method has been used almost exclusively in adult (8 months old) Brown Norway rats maintained in a constant light environment.⁶ Observations by the Morrison group had suggested that animals that undergo hypertonic saline sclerosis show significant enlargement of the experimental eye, consistent with direct obstruction of aqueous humor outflow.⁶ In contrast, in preliminary observations we did not find a significant difference between experimental and control eyes in the EVC animals, suggesting that a different mechanism of pressure elevation occurs in this model. Because it has also been reported recently that the EVC model may have significant variability in the amount of retinal ganglion cell (RGC) loss, with a significant number of animals not displaying damage,^{1,17} we decided to investigate whether there are indeed quantitative changes that occur in the AC of the eye that may account for these differences between the two models. Although a crossover design (where induction of elevated IOP is caused in both strains—Wistar and Brown Norway—by both methods) under identical conditions would be ideal, the investment in both time and cost made such a study difficult to perform. In addition, issues like IOP measurement in nonanesthetized animals (which is impossible in the nondocile Wistar rats) and the knowledge that hypertonic saline sclerosis is not as effective in Wistar rats (Morrison J, unpublished observation, 2002) made such a design impractical. In view of these limitations, we elected to compare the two models in the best possible light for each one and in the strain in which each is most commonly reported. This study represents the collaborative effort of two laboratories (the Morrison/Johnson and the Mittag/Danias laboratories) in an effort to try to reconcile some of the findings reported in the literature.

UBM has been used to characterize and describe the AC anatomy in many species, including the rat.¹² Its resolution, as well as the depth of imaging, depends on the frequency of the ultrasonic transducer used. For the 50-MHz transducer used in this study, axial and lateral resolution is approximately 50 μm , and the depth of penetration is up to 5 mm. This resolution and penetration depth provides the best possible balance for the small rat eye but undoubtedly introduces some uncertainty in some of the measurements.

The two groups of rats studied were generated by investigators who have significant expertise in each particular model. The animals were maintained under different conditions (as previously described in the literature for each of the models) but were transported to one laboratory (in New York) for UBM imaging before death. IOP histories were in agreement with values previously reported in the literature. Surprisingly, a direct comparison of maximum IOP, mean IOP, and cumulative IOP exposure for the two strains revealed that the EVC group had greater IOP differences between experimental and control eyes. This difference may in part be accounted for by the fewer IOP measurements performed and the fact that animals were lightly sedated in this group. As a consequence, control eyes in the EVC group had IOPs in the high teens, whereas control eyes in the HSEVS group had IOPs in the high 20s. Thus, the pressure differential between experimental and control eyes could actually overestimate the absolute pressure response in the episcleral cautery group compared with the hypertonic saline group. As previously reported, animals that underwent EVC showed elevation of IOP very early after the procedure. In contrast, episcleral vein sclerosis causes a delayed IOP elevation approximately 10 days after the procedure.

Control eyes in the two groups showed a significant difference in most of the parameters studied, except for the ACD. This is understandable, given the differences in strain and age

between the two groups. However, these differences may also explain why the 8-month-old Brown Norway rats may be more appropriate for a hypertonic saline injection glaucoma model. These animals appear to have significantly narrower angles and thicker irides which would create an anatomic predisposition for the development of synechial closure when sclerotic agents flow through the TM. Although we did not detect a statistically significant difference in the angle anatomy between experimental and control eyes in the Brown Norway rats, small peripheral anterior synechiae (PAS) that would occlude the TM would not be discerned, given the resolution limitations of our methods.

Quantitative UBM imaging of experimental eyes confirmed our prior qualitative observations that AC structures do not change in the EVC model, whereas they do so in the HSVES injection model. The most dramatic such change was the deepening of the AC observed in the Brown Norway rats, where the average ACD almost doubled in depth. Whether this actually represents enlargement of the eye or is caused by a backward movement of the iris–lens diaphragm is unclear. Figure 3 suggests that both of these mechanisms may be operational. We interpret the increased AC depth as strong evidence that the increase in IOP after hypertonic saline injection is due to the obstruction of aqueous humor outflow and is consistent with the histologic evidence of trabecular scarring presented with the initial description of this method.⁶ The distinct absence of AC deepening in the cautery group, which had an equal or greater increase in IOP, implies that some other mechanism is responsible for the elevation of IOP in this model.

In addition, in the HSVES experimental eyes, the ciliary body appeared to decrease in size compared with that of the contralateral control eye. This decrease may correspond to atrophy and could represent either a direct effect of the hypertonic saline on the ciliary body or the effect of increased IOP in the ciliary processes. The fact that higher IOP did not result in similar changes in the EVC group makes the first explanation more plausible, but again the age of the animals and the difference in strain may mitigate such comparisons.

The changes in ACD correlated well with IOP history (maximum IOP, mean IOP, and cumulative IOP exposure) in the Brown Norway rats. In contrast, the correlation of ciliary body size with IOP was not as strong, although still significant. The presence of debris in the AC of some experimental eyes in the hypertonic saline injection group suggests the presence of a mild inflammation. This inflammatory response may be partly responsible for scarring and occlusion of the aqueous outflow pathways (indicated by the deeper AC and by the fact that IOP does not tend to decrease significantly with time in this model; Morrison J, et al., unpublished observations, 2002). This potential scenario is also consistent with the observation that IOP generally does not increase until several days after injection.⁶ This delay may result from the simultaneous development of reduced inflammation, recovery of ciliary body function and increased aqueous production along with gradual scarring of the AC angle.

In summary, our findings indicate that these two popular glaucoma models in rats differ in their effects on the AC structures and likely mechanism of IOP elevation. Prior work^{1,15,17} has shown that the two models also differ in their consistency and extent of causing RGC loss. Thus, these two models are not interchangeable, and results obtained with one are not necessarily applicable to the other. Selection of the most appropriate model for future investigations depends on the type of study planned.

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FIGURE 1.
IOP history of the two groups of animals used in the study.

FIGURE 2.
UBM images from experimental (A, C) and contralateral control (B, D) eyes of rats in the EVC group.

FIGURE 3. UBM images from experimental (**A, C**) and contralateral control (**B, D**) eyes of rats in the HSEVS group. Notice the significantly deeper AC and flatter iris contour in experimental eyes.

FIGURE 4.
Correlation of AC depth with IOP in the HSEVS group of rats.

FIGURE 5.
Correlation of ciliary body size with IOP in the HSEVS group of rats.

FIGURE 6.
UBM image from a rat in the HSEVS group with significant inflammatory debris in the AC.

Table 1

Parameters Measured in Control (Nonsurgical) Eyes for Both Strains of Rats

	Brown Norway (<i>n</i> = 12)	Wistar (<i>n</i> = 10)	<i>P</i>
ACD (μm)	778.5 ± 42.7	813.5 ± 45.0	0.43 NS
TIA (degrees)	14.8 ± 7.2	23.0 ± 5.8	0.002
IT PM (μm)	256.6 ± 37.9	192.8 ± 15.2	0.00007
AOD (μm)	45.9 ± 17.3	126.1 ± 33.0	0.000001
CB (μm ²)	408.1 ± 87.0	604.3 ± 97.8	0.00007

Control values (mean ± SD) for the Wistar rats were calculated for 4 to 5 weeks after surgery in the group that underwent EVC.

Table 2

Parameters Measured in Control and Experimental Eyes in the 4- to 5-Week EVC Group of Rats

	Control	Experimental	<i>P</i>
ACD (μm)	813.5 $\pm$ 45.0	851.1 $\pm$ 64.0	0.15 NS
TIA (degrees)	23.0 $\pm$ 5.8	23.4 $\pm$ 5.4	0.88 NS
IT PM (μm)	192.8 $\pm$ 15.2	199.0 $\pm$ 12.6	0.19 NS
AOD (μm)	126.1 $\pm$ 33.0	119.7 $\pm$ 26.8	0.60 NS
CB (μm^2)	604.3 $\pm$ 97.8	544 $\pm$ 82.8	0.17 NS

Data are the mean $\pm$ SD.

Table 3

Parameters Measured in Control and Experimental Eyes in the 4- to 5-Week HSEVS Group of Rats

	Control	Experimental	<i>P</i>
ACD (μm)	778.5 ± 42.7	1347 ± 200.1	<0.000001
TIA (degrees)	14.8 ± 7.2	17.4 ± 7.6	0.28 NS
IT PM (μm)	256.6 ± 37.9	274.9 ± 37.1	0.30 NS
AOD (μm)	45.9 ± 17.3	60.2 ± 25.1	0.11 NS
CB (μm ²)	408.1 ± 87.0	278.4 ± 66.0	0.0002

Data are the mean ± SD.