

Effects of Dorzolamide on Retinal and Choroidal Blood Flow in the DBA/2J Mouse Model of Glaucoma

Saurav Chandra, Eric R. Muir, Kaiwalya Deo, Jeffrey W. Kiel, and Timothy Q. Duong

Department of Ophthalmology and Research Imaging Institute, University of Texas Health Science Center, San Antonio, Texas, United States

Correspondence: Timothy Q. Duong, University of Texas Health Science Center at San Antonio, Department of Ophthalmology, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA; duongt@uthscsa.edu.

Submitted: September 28, 2015

Accepted: January 25, 2016

Citation: Chandra S, Muir ER, Deo K, Kiel JW, Duong TQ. Effects of dorzolamide on retinal and choroidal blood flow in the DBA/2J mouse model of glaucoma. *Invest Ophthalmol Vis Sci*. 2016;57:826–831. DOI:10.1167/iov.15-18291

PURPOSE. To test the hypothesis that acute topical dorzolamide (DZ) decreases intraocular pressure (IOP) and increases retinal and choroidal blood flow in the DBA/2J mouse model of glaucoma.

METHODS. Retinal and choroidal blood flow were measured in 4- and 9-month-old DBA/2J mice, and 4-month C57BL/6 (control) mice under isoflurane anesthesia using magnetic resonance imaging. Ocular blood flow was measured at baseline, and 1 and 2 hours after topical dorzolamide. Intraocular pressure was measured using a rebound tonometer in a subset of animals at the same time points.

RESULTS. Baseline IOP in the 4-month-old DBA/2J mice and C57BL/6 mice was not significantly different ($P > 0.05$), and IOP in both groups was less than in the 9-month-old DBA/2J mice ($P < 0.05$ for both). Compared to baseline, dorzolamide reduced IOP at 1 and 2 hours after dorzolamide in the 4- ($P < 0.05$) and 9-month-old ($P < 0.01$) DBA/2J mice, but not in the C57BL/6J mice ($P > 0.05$). Baseline retinal blood flow was lower in the 4-month and 9-month-old DBA/2J mice compared with the 4-month-old C57BL/6J mice ($P < 0.05$). Baseline choroidal blood flow in the 9-month-old DBA/2J mice was less than in the C57BL/6J mice ($P < 0.05$). Compared with baseline, both retinal and choroidal blood flow increased at 1-hour post-dorzolamide and remained elevated 2 hours later in the 9-month-old DBA/2J mice ($P < 0.05$).

CONCLUSIONS. Dorzolamide lowers IOP and raises retinal and choroidal blood flow in older DBA/2J mice, consistent with the study hypothesis.

Keywords: MRI, regional blood flow, glaucomatous optic neuropathy

Animal models often utilize ocular hypertension to study glaucoma pathogenesis, altered homeostatic mechanisms, and pharmacology.¹ One widely used model is the DBA/2J mouse.^{2–6} The mouse DBA/2J has altered expression of two proteins: tyrosinase-related protein 1 associated with iris stromal atrophy, and glycoprotein transmembrane NMB associated with iris pigmentary dispersion, which raises intraocular pressure (IOP) by blocking the trabecular meshwork and reducing outflow facility.^{7,8} The resultant ocular hypertension is associated with glaucoma-like retinal ganglion cell (RGC) loss with age.

We previously used the “arterial spin labeling” magnetic resonance imaging (ASL-MRI) technique to measure retinal and choroidal blood flow (RBF and ChBF, respectively) in the DBA/2J mouse model.⁹ We found that RBF and ChBF were reduced in DBA/2J mice compared to C57BL/6 control mice, suggesting a possible ischemic contribution to the optic neuropathy and visual dysfunction that occur in older DBA/2J mice.^{10–18} Interestingly, there is evidence that lowering the IOP pharmacologically enhances RGC survival in older DBA/2J mice.^{19,20} It is unknown whether lowering IOP would increase their ocular blood flow, which might contribute to the enhanced RGC survival. Therefore, to further characterize the DBA/2J model, the goal of the present study was to test the hypothesis that lowering IOP increases RBF and ChBF in the DBA/2J mouse at different ages. To lower IOP, topical dorzolamide (DZ) was

chosen because it inhibits aqueous humor production²¹ and it was assumed that an aqueous suppressant would be more effective at lowering IOP than a drug that enhances outflow facility (e.g., pilocarpine) since the ocular hypertension in the DBA/2J model is due to obstruction of the trabecular outflow pathway.

METHODS

The protocol was approved by the local Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

MRI Measurements of Blood Flow (BF)

Three groups of animals were used for MRI study: male wild-type C57BL/6J mice aged 4 months ($n = 8$), and male DBA/2J mice aged 4 months ($n = 8$) and 9 months ($n = 6$). During imaging, mice spontaneously breathed a mixture of 30% O₂/70% N₂ with 1.6% isoflurane for anesthesia while the animals were held in a custom-made holder with ear and tooth bars to minimize motion. Respiratory rate was monitored via a force transducer and maintained in a target range of 80 to 120 breaths per minute by slight adjustments to the level of anesthesia.



Heart rate and arterial oxygen saturation were also monitored using oximetry (MouseOx; STARR Life Science Corp., Oakmont, PA, USA). Animal temperature was monitored and maintained at 37°C with warm water that circulated through a water pad underneath the mouse throughout the experiment.

Blood flow MRI was acquired at baseline (before DZ application). Then a single drop (5 μ L) of dorzolamide HCL ophthalmic solution (2%, Bausch and Lomb) was applied on the left eye, and BF MRI was acquired again at 1 and 2 hours after DZ application. The animals were allowed to recover after the scans were completed.

MRI Parameters

Depth-resolved BF MRI was performed at a resolution of $42 \times 42 \times 400 \mu\text{m}$ in a magnet with a 150 Gauss/cm gradient (Bruker Biospec 7 Tesla; Bruker Corp., Billerica, MA, USA) using a custom circular eye coil for imaging (diameter = 6 mm) and circular heart coil for ASL (inner diameter = 8 mm).^{22,23} The blood flow scans were acquired with a gradient-echo, echo-planar imaging sequence with a $6 \times 6 \text{ mm}$ field of view and 144×144 matrix ($42 \times 42 \mu\text{m}$ resolution in-plane) zero-filled interpolation to 256×256 . The blood flow sequence used a single, 400 μm coronal slice, two shots, 2.94-second labeling pulse, 3.0-second repetition time, and a 13 ms echo time. The slice was positioned near the optic nerve and tilted perpendicular to the retina. Blood flow values were calculated from images acquired over a 20-minute period and averaged offline.

Image analysis was performed with custom software (MATLAB; MathWorks, Inc., Natick, MA, USA), and STIMULATE (University of Minnesota, www.cmrr.umn.edu) software packages as described in detail elsewhere.²² A semiautomated process in (MathWorks, Inc.) was used to linearize the retina; align the retina to correct for motion (if any) of the eye during the scan; and conduct an automated profile analysis. Profiles across the retinal thickness were obtained from images by projecting lines perpendicular to the retina with profiles obtained at $\times 4$ spatial interpolation. The blood flow (mL/min/g) was calculated from the signal intensities of labeled and nonlabeled images as: $\text{BF} = (\lambda / T_1)(S_{\text{NL}} - S_L) / (S_L + [2\alpha - 1]S_{\text{NL}})$, where λ (0.9 mL/g) is the tissue-blood partition coefficient for water and is the value ([quantity of water/grams of tissue] / [quantity of water/mL of blood]); T_1 is 1.8 seconds at 7 Tesla, S_{NL} is the signal intensity (arbitrary units) of images with non-labeled blood, S_L (arbitrary units) is the signal intensity of images with magnetically labeled blood, and α is the arterial spin-labeling efficiency (0.7) for cardiac labeling in mice. Blood flow profiles were averaged along the retina-choroid complex. Two peaks were present in the averaged BF profile, located in the inner retina and choroid. Measurements of retinal and choroidal BF were determined from the corresponding peaks of the average BF profiles for each animal. The arterial spin labeling MRI method to measure retinal and choroid blood flow has been corroborated with the microsphere technique.²⁴

Intraocular Pressure (IOP) Measurements

Measurements of IOP were performed with a rebound tonometer (Icare Tonolab, Helsinki, Finland)^{25,26} on both eyes of 4-month-old C57BL/6J ($n = 3$), 4-month-old DBA/2J ($n = 3$), and 9-month-old DBA/2J ($n = 3$) male mice. These measurements were taken on a separate occasion than the MRI scans. The animals were anesthetized with 1.6% isoflurane and body temperature was maintained at 37°C using a heating pad. An average of six readings were taken at baseline, and again at 1 and 2 hours post-DZ applications. A single drop (5 μ L) of dorzolamide HCL ophthalmic solution (2%, Bausch and Lomb)

was applied on each eye. The animals were allowed to recover after the IOP measurements were completed. The same protocol was followed for an additional group of 9-month-old DBA/2J male mice ($n = 3$) that received topical saline as a control.

Arterial Pressure Measurements

We previously found that arterial pressure in older (age >6 months) C57BL/6 ($n = 9$) and DBA/2J ($n = 9$) mice was stable for 1 hour under isoflurane anesthesia.⁹ To confirm that blood pressure remained stable during the 2-hour period of anesthesia in the present study, femoral arterial pressure was measured in a 6-month-old C57BL/6 mouse and a 9-month-old DBA/2J mouse under 1.6% isoflurane. A femoral artery cutdown was performed and arterial pressure in the femoral artery was measured for >2 hours using a servo-null micro-pressure system (Model 900A; World Precision Instruments, Sarasota, FL, USA) and a recording system (PowerLab; ADInstruments, Colorado Springs, CO, USA).⁹ The technique uses glass micropipettes drawn to a 3 to 5 μm diameter tip to cannulate the target blood vessel. The pipette is filled with 2M NaCl that permits an electric circuit to be established between the pipette and a reference electrode placed in the tissue nearby. The resistance across the pipette tip is monitored and a pressure pod with a fast piezoelectric valve applies the necessary pressure to the open end of the pipette to maintain the resistance constant at the tip in the vessel. The applied pressure is taken to be equivalent to the pressure in the vessel. After the experiment, the mice were euthanized without regaining consciousness.

Statistical Analysis

The intraocular pressure data for each animal were pooled (i.e., the six measurements in each eye were averaged and then the mean IOP for the two eyes in each animal was averaged to give a single IOP value per animal). All data are reported as mean \pm standard error of the mean, with significant differences accepted at $P < 0.05$. Comparisons within strain by time point were done by 1-way repeated measures ANOVA with Bonferroni post hoc tests. Comparisons between strains by time point were done by 2-way repeated measures ANOVA with Bonferroni post hoc tests (GraphPad Prism, La Jolla, CA, USA).

RESULTS

IOP

Figure 1 shows the IOP measurements at baseline, and 1 and 2 hours after topical DZ application outside the MRI scanner but under otherwise identical conditions as in the MRI experiments. Baseline IOP in the 9-month-old DBA/2J ($22.7 \pm 0.2 \text{ mm Hg}$) mice was significantly higher than in the C57BL/6J ($11.4 \pm 0.6 \text{ mm Hg}$, $P < 0.001$) and 4-month-old DBA/2J ($11.0 \pm 1.4 \text{ mm Hg}$, $P < 0.001$) mice. Baseline IOP was not significantly different between C57BL/6J and 4-month DBA/2J ($P > 0.05$), or between 9-month-old DBA/2J mice that received DZ or saline ($21.5 \pm 1.5 \text{ mm Hg}$, $P > 0.05$). One hour after DZ application, the IOP in the 4 DBA/2J ($7.5 \pm 0.4 \text{ mm Hg}$) and 9-month-old DBA/2J ($7.8 \pm 0.6 \text{ mm Hg}$) mice was significantly lower than baseline ($P < 0.05$ and $P < 0.01$, respectively) and remained significantly reduced ($P < 0.05$ and $P < 0.01$, respectively) compared with baseline at 2 hours after DZ (7.5 ± 0.8 and $8.0 \pm 0.15 \text{ mm Hg}$, respectively). The intraocular pressure in the C57BL/6J mice was not significantly different ($P > 0.05$) from baseline at 1 or 2 hours after DZ (8.8 ± 0.2

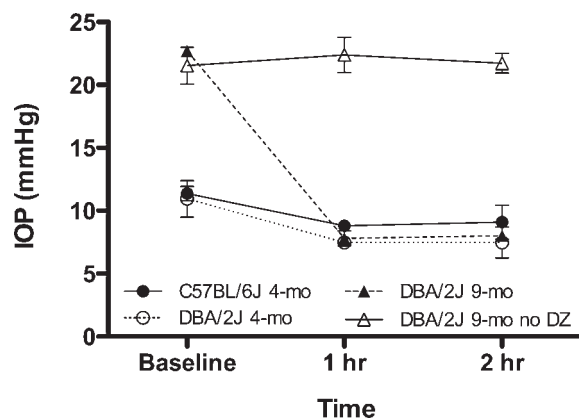


FIGURE 1. Mean IOP measurements for 4-month-old C57BL/6J, 4-month-old DBA/2J, and 9-month-old DBA/2J mice at baseline and at 1 and 2 hours post-DZ as well as 9-month-old DBA/2J mice before and after topical saline. See text for description of statistically significant differences.

and 9.1 ± 0.9 mm Hg, respectively). The intraocular pressure also was not significantly different ($P > 0.05$) from baseline at 1 and 2 hours after saline instead of DZ in 9-month-old DBA/2J mice (22.4 ± 1.4 and 21.72 ± 0.8 mm Hg, respectively).

MRI of Blood Flow

Figure 2A shows a layer-specific blood flow image from a normal mouse eye. Figure 2B displays an example of RBF and ChBF profiles in one control eye. The choroidal BF is markedly higher than the RBF, and the middle avascular layer has a minimal blood flow signal as expected.

Retinal BF and ChBF were measured at baseline, and 1 and 2 hours post-DZ application (Fig. 3). Baseline RBF was significantly lower in both the 4- (0.90 ± 0.08 mL/min/g) and 9-month-old (0.78 ± 0.05 mL/min/g) DBA/2J mice compared with the C57BL/6J mice (1.24 ± 0.11 mL/min/g). At 1 hour after DZ, RBF was significantly increased ($P < 0.05$) in the 9-month-old DBA/2J mice (1.17 ± 0.03 mL/min/g), but not in the C57BL/6J (1.13 ± 0.06 mL/min/g) or the 4-month-old DBA/2J mice (1.06 ± 0.05 mL/min/g). At 2 hours after DZ, RBF

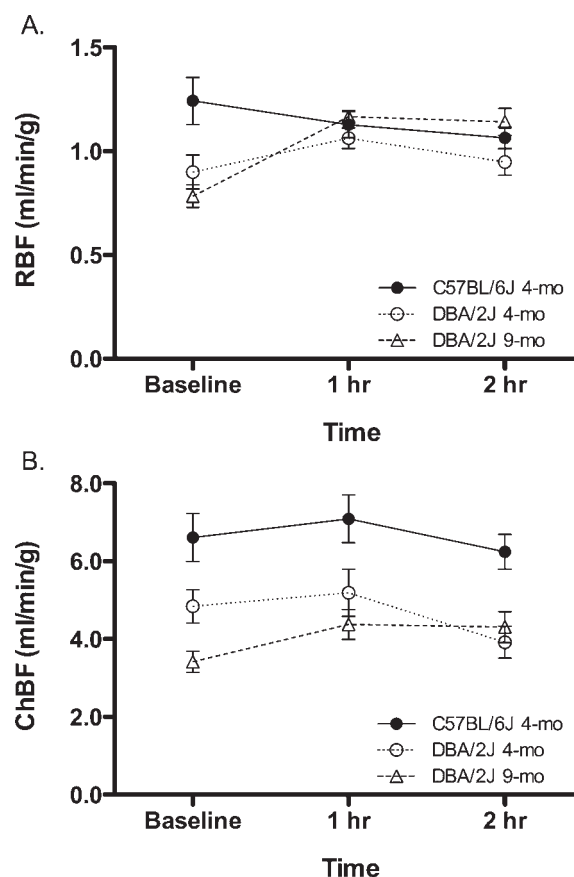


FIGURE 3. Retinal (A) and choroidal BF (B) at baseline, and 1 and 2 hours after topical dorzolamide in 4-month-old C57BL/6J, 4-month-old DBA/2J, and 9-month-old DBA/2J mice. See text for description of statistically significant differences.

in the 9-month-old DBA/2J (1.14 ± 0.07 mL/min/g) remained elevated above baseline ($P < 0.05$). Although trending lower, RBF in the C57BL/6J (1.06 ± 0.05 mL/min/g) and 4-month-old DBA/2J (0.95 ± 0.07 mL/min/g) mice remained unchanged from baseline at 2 hours after DZ.

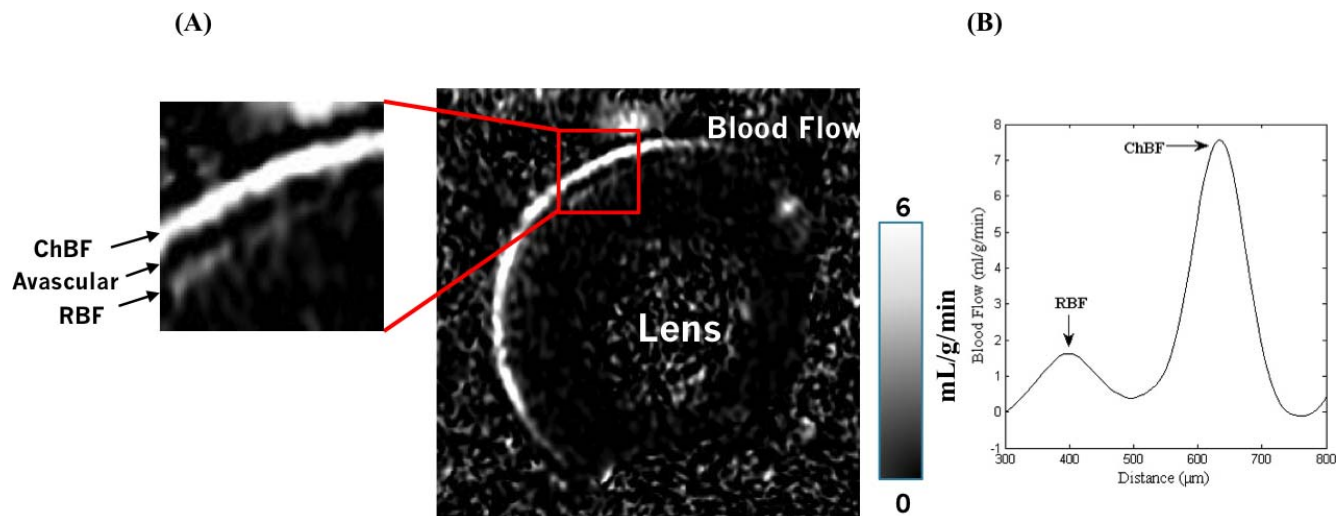


FIGURE 2. (A) Layer-specific blood flow map of a C57BL/6J mouse eye at a resolution of $42 \times 42 \times 400$ μm . Blood flow maps detect choroidal and retinal vascular layers with the avascular zone in between. Scale bar: indicates the blood flow range. (B) Retinal and choroidal blood flow profiles (mL/min/g) were calculated from the signal intensities of labeled images such as in Figure 2A.

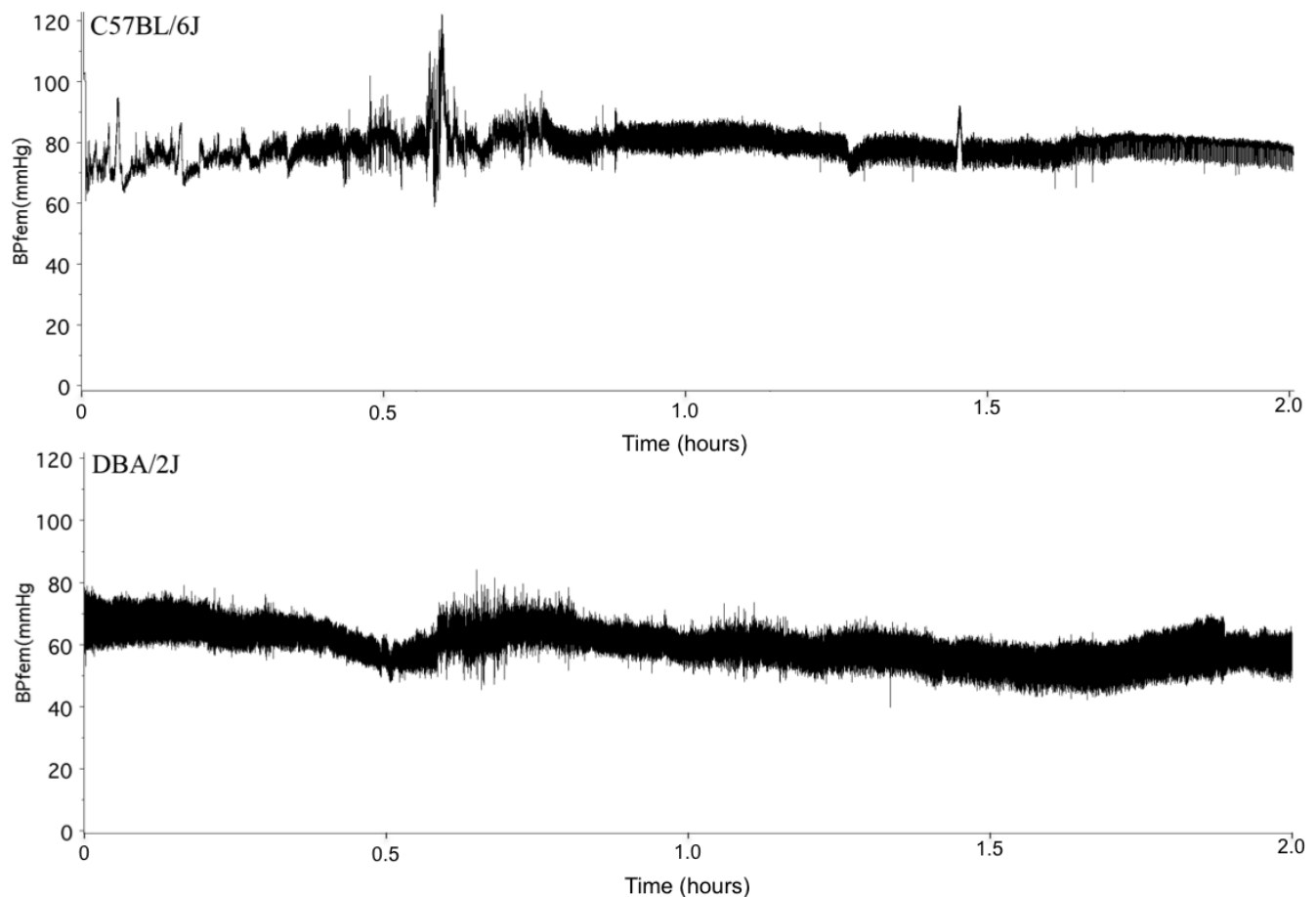


FIGURE 4. Arterial pressure traces from a 6-month-old C57BL/6J mouse (*top*) and a 9-month-old DBA/2J mouse (*bottom*) over a 2-hour period of anesthesia similar to that used in the MRI experiments table.

Baseline ChBF was significantly lower in the 9-month-old DBA/2J mice (3.42 ± 0.27 mL/min/g) compared with the C57BL/6J mice (6.61 ± 0.62 mL/min/g) and the 4-month-old DBA/2J mice (4.84 ± 0.43 mL/min/g). At 1 hour after DZ, ChBF significantly increased ($P < 0.05$) in the 9-month-old DBA/2J mice (4.38 ± 0.39 mL/min/g), but not in the C57BL/6J (7.09 ± 0.62 mL/min/g) and the 4-month-old DBA/2J (5.19 ± 0.61 mL/min/g) compared with baseline. At 2 hours after DZ, ChBF remained above baseline ($P < 0.05$) in the 9-month-old DBA/2J mice (4.31 ± 0.40 mL/min/g); however, ChBF in the C57BL/6J (6.24 ± 0.45 mL/min/g) and the 4-month-old DBA/2J (3.92 ± 0.41 mL/min/g) mice were not significantly different from baseline, although both decreased significantly ($P < 0.05$) from their 1 hour values. Choroidal BF in the 9-month-old DBA/2J mice was lower than in the C57BL/6J mice at all time points ($P < 0.05$).

Blood Pressure Measurements

Systemic blood pressure in a 6-month-old C57BL/6J and a 9-month-old DBA/2J mouse were measured outside the MRI scanner over a similar 2-hour period as the MRI experiments (Fig. 4). The blood pressure was relatively stable in both animals, averaging (mean \pm SD) 79 ± 5 mm Hg in the C57BL/6J mouse and 60 ± 5 mm Hg in the DBA/2J mouse, consistent with our previous experience with these mouse strains for 1 hour under anesthesia.⁹

DISCUSSION

Ocular hypertension and the consequent diminished perfusion pressure available to drive blood through the ocular circulation is the basis for the longstanding ischemic hypothesis of glaucoma. The present study and other investigations of the DBA/2J model support this hypothesis since it develops age-related ocular hypertension, reduced ocular blood flow and the RGC loss and decreased optic nerve axon density seen in human glaucoma. The present study adds to the existing literature by showing that acute pharmacologic IOP reduction with topical dorzolamide in the older DBA/2J mouse is associated with increased retinal and choroidal blood flow.

Dorzolamide is a carbonic anhydrase inhibitor commonly used in glaucoma treatment to lower IOP.²⁷ Its hypotensive effect is mediated by inhibition of aqueous humor production.²¹ In addition to its hypotensive effect, dorzolamide dilates isolated retinal arterioles^{28,29} and so it may have a direct vasodilatory effect in vivo. However, although topical dorzolamide reaches the back of the eye,^{30,31} its ability to increase blood flow in the retina, choroid and optic nerve has not been shown consistently. In rabbits, acute dorzolamide had no effect on choroidal perfusion,³² but chronic dosing increased optic nerve head perfusion in one study³³ and had no effect in another.³⁴ For humans, some studies reported no change in retinal blood flow^{35–37} while others reported an increase.^{38–41} Similarly, for human optic nerve head blood flow, dorzolamide was reported to have no effect⁴² or to increase perfusion⁴³ and

to flatten the pressure-flow relationship.⁴⁴ For human choroidal blood flow, modest increases in perfusion indexes were reported.^{43,45} The discrepancies in the literature are likely due to differences in dosing protocols, and also the inherent ambiguities in the methods used to measure blood flow in the human ocular circulations. However, on balance, it does not appear that dorzolamide has a dramatic hyperemic effect on human retinal or choroidal blood flow.

What then accounts for the blood flow responses in the present study? As ocular blood flow could be affected by instability of systemic blood pressure over the course of the 2-hour MRI experiments, we monitored arterial blood pressure over the same period outside the MRI scanner. We found the arterial blood pressure to be stable over 2 hours under isoflurane anesthesia, consistent with our previous results in larger cohorts of DBA/2J and C57BL/6J ($n = 9$ each) during 1 hour of isoflurane anesthesia.⁹ Thus, the changes in ocular blood flow after DZ do not seem to be due to systemic blood pressure instability. By contrast, IOP in the older DBA/2J group was markedly elevated at baseline and declined markedly after dorzolamide. The perfusion pressure ranges for retinal and choroidal pressure-flow autoregulation in the DBA/2J mouse are unknown; however, the perfusion pressure below which retinal and choroidal blood flow respond linearly to perfusion pressure changes is approximately 40 mm Hg in several species.⁴⁶ Assuming the perfusion pressure was below the autoregulatory cut-off pressure in the older DBA/2J mice at baseline, it is plausible that the increase in perfusion pressure when IOP decreased after DZ would cause the observed increases in retinal and choroidal blood flow.

For the younger DBA/2J group and the C57BL/6J group, the interpretation of the retinal and choroidal blood flow response pattern is less clear. At baseline, their IOPs were similar and relatively low, and did not fall markedly after dorzolamide. Thus, IOP does not explain the reduced baseline retinal blood flow and marginally reduced ($P = 0.07$ by unpaired t -test) choroidal blood flow in the younger DBA/2J group, nor does IOP explain the fall in choroidal blood flow in the second hour after dorzolamide. The differences in baseline retinal and choroidal blood flows may be due to arterial pressure. Arterial pressures in conscious C57BL/6J and DBA/2J mice are similar and around 100 mm Hg.^{47,48} Under isoflurane anesthesia, arterial pressure may fall lower in DBA/2J mice than C57BL/6J mice, as suggested by the two animals we tested (Fig. 4); higher IOP and low arterial pressure would obviously contribute to the even lower baseline retinal and choroidal blood flows in the older DBA/2J mice. However, the fall in choroidal blood flow in the DBA/2J and C57BL/6J during the second hour after dorzolamide cannot be explained by perfusion pressure. A possible explanation is washout of an endogenous vasodilator during the first hour hyperemia, but this is speculative.

CONCLUSIONS

This study investigated the effects of acute topical application of dorzolamide on IOP and retinal and choroidal blood flow in an animal model of glaucoma. The results support the hypothesis that acute topical dorzolamide decreases intraocular pressure and increases retinal and choroidal blood flow in the older DBA/2J mouse model of glaucoma. Future studies will investigate the effects of chronic dorzolamide treatment on retinal and choroidal blood flow in young and old DBA/2J mice.

Acknowledgments

Supported by the NIH (EY021173); a MERIT award from the Department of Veterans Affairs, a Translational Resource Technol-

ogy grant and a Pilot grant from the Clinical Translational Science Award (CTSA, parent grant UL1RR025767); and the van Heuven endowment.

Disclosure: **S. Chandra**, None; **E.R. Muir**, None; **K. Deo**, None; **J.W. Kiel**, None; **T.Q. Duong**, None

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