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Effects of Dorzolamide on Choroidal Blood Flow, Ciliary Blood Flow, and Aqueous Production in Rabbits

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Abstract

Purpose—To determine the effects of topical dorzolamide (a carbonic anhydrase inhibitor) on choroidal and ciliary blood flow and the relationship between ciliary blood flow and aqueous flow.

Methods—The experiments were performed in four groups of pentobarbital-anesthetized rabbits treated with topical dorzolamide (2%, 50 μ L). In all groups, intraocular pressure (IOP) and mean arterial pressure (MAP) at the eye level were measured continuously by direct cannulation. In group 1, aqueous flow was measured by fluorophotometry before and after dorzolamide treatment. In group 2, aqueous flow was measured after dorzolamide at normal MAP and while MAP was held constant at 80, 55, or 40 mm Hg with occluders on the aorta and vena cava. In group 3, the same MAP levels were used, and ciliary blood flow was measured transsclerally by laser Doppler flowmetry (LDF). In group 4, choroidal blood flow was measured by LDF with the probe tip positioned in the vitreous over the posterior pole during ramp increases and decreases in MAP before and after dorzolamide.

Results—Dorzolamide lowered IOP by 19% ($P < 0.01$) and aqueous flow by 17% ($P < 0.01$), and increased ciliary blood flow by 18% ($P < 0.01$), which was associated with a significant reduction in ciliary vasculature resistance (-7% , $P < 0.01$). Dorzolamide shifted the relationship between ciliary blood flow and aqueous flow downward relative to the previously determined control relationship in the rabbit. Dorzolamide did not alter choroidal blood flow, choroidal vascular resistance, or the choroidal pressure flow relationship.

Conclusions—Acute topical dorzolamide is a ciliary vasodilator and has a direct inhibitory effect on aqueous production, but it does not have a detectable effect on choroidal hemodynamics at the posterior pole in the rabbit.

Carbonic anhydrase inhibitors (CAIs) are commonly used in the treatment of glaucoma and ocular hypertension.¹ When introduced in the 1950s, the ophthalmic community was interested in the IOP-lowering effect and then later started focusing on the vascular effects of CAIs.^{2,3} Although the ocular hypotensive effect of CAIs is undisputed, their effects on ocular blood flow are more ambiguous.^{4,5} Many of the CAI ocular blood flow studies examined the effects of the topically administered CAI dorzolamide,^{6–8} and those results are

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contradictory, with some studies indicating no or minor effects on ocular blood flow,^{9–14} whereas other studies report significant effects on ocular hemodynamics.^{15–18} There is also evidence that dorzolamide increases oxygen concentration in the optic nerve head and in the retina, which may be caused by a reduction in local metabolism or an increase in blood flow.^{19,20} In vitro studies also show a direct vasodilatory effect of CAIs on retinal arterioles.^{21,22} Given the ambiguity in the reported ocular blood flow responses to dorzolamide, one goal of the present study was to determine its effects on choroidal and ciliary blood flow at different perfusion pressures in an established rabbit model,^{23,24} which had not been done previously.

The second goal of the study was to determine the effect of dorzolamide on the relationship between ciliary blood flow and aqueous production. Under control conditions, aqueous production is relatively constant until ciliary blood flow is reduced 20% to 30% below the control if ciliary blood flow is varied over a wide range by mechanical manipulations of blood pressure. With further reductions in ciliary blood flow, aqueous production decreases in a blood flow–dependent manner.²⁵ Drugs that cause ciliary vasoconstriction and lower ciliary blood flow below the critical perfusion level decrease aqueous production to the same extent as mechanical blood flow reductions, indicating those drugs decrease aqueous production indirectly by depriving the ciliary epithelium of the blood flow needed to sustain ciliary metabolism.²⁶ Dorzolamide decreases IOP by decreasing aqueous production, presumably by limiting bicarbonate availability.^{27–29} We hypothesized that dorzolamide would shift the blood flow–independent portion of the curve relating ciliary blood flow and aqueous production downward, consistent with a direct inhibitory effect on ionic transport within the cells of the ciliary epithelia.

Methods

The animal procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. At the end of the experiment, all animals were euthanatized with an overdose of anesthetic without ever regaining consciousness.

Animal Preparation

A total of 76 New Zealand albino rabbits (2.2 ± 0.4 kg) of both sexes were used in this study. The animals were housed for 1 to 3 days in the vivarium with food and water available ad libitum. The animals were anesthetized with pentobarbital sodium (30 mg/kg, IV, supplemented as needed), paralyzed with gallamine triethiodide (1 mg/kg) to eliminate eye movement, and intubated through a tracheotomy and respired with room air. Expired P_{CO_2} was monitored (Normocap 200; Datex, Tewksbury, MA) and maintained at ≈ 40 mm Hg. A heating pad was used to maintain normal body temperature ($38\text{--}39^\circ\text{C}$). All intravenous injections were given via cannulated marginal ear veins.

To estimate the ocular mean arterial pressure (MAP), we inserted a catheter into the right ear artery and connected it to a pressure transducer positioned at the same height above the heart as the eye. To set the MAP at the specified target pressures, we placed hydraulic occluders around the thoracic descending aorta and the inferior vena cava through a right thoracotomy.

The aortic occluder was used to redirect the cardiac output to the upper body and thus to increase the MAP at the eye. The caval occluder was used to impede venous return, thereby lowering cardiac output and reducing MAP throughout the body.

After the initial surgical preparation, the animals were mounted in a stereotaxic head holder. In most animals ($n = 51$), the orbital venous sinus was cannulated with a 23-gauge needle inserted through the posterior supraorbital foramen to measure orbital venous pressure (OVP) with a second pressure transducer. (The necessary equipment to record OVP was unavailable for the other 25 animals.) In all animals, the right eye was cannulated with a 23-gauge needle inserted into the vitreous cavity through the pars plana to measure the IOP with a third pressure transducer. To avoid the rabbit ocular trauma response and release of prostaglandins, the right eye was anesthetized topically with lidocaine before the cannulation and care was taken not to disturb the cornea and anterior chamber.^{30–32}

Aqueous Flow Measurement

Aqueous flow was measured by fluorophotometry (FM-2; OcuMetrics, Mountain View, CA). Each animal received 4 drops of fluorescein (2.5 mg/mL, Flurox; Ocusoft, Richmond, TX) at ≈ 8 AM on the day of the experiment. Two hours later, the animals were anesthetized, the treated eye irrigated with saline to remove excess fluorescein, and the animal prepared as described earlier. Once the animals were mounted in the stereotaxic instrument and stable (3–3.5 hours after fluorescein application), triplicate fluorophotometric scans were performed at 15-minute intervals to measure the changes in corneal and anterior chamber fluorescein concentrations over time. Aqueous flow was calculated based on Brubaker's method after applying the focal diamond correction to the raw corneal fluorescein concentration data.^{33,34}

Ciliary and Choroidal Blood Flow Measurement

Laser Doppler flowmetry (LDF) was used to measure ciliary and choroidal blood flow. LDF provides three indices of perfusion derived from the frequency spectra collected from tissue illuminated with laser light: the number of moving blood cells, their mean velocity, and the flux (the product of the velocity and number of moving blood cells). The flux has been shown to correlate linearly with independent measures of blood flow in a variety of tissues. A detailed description of LDF and its validation are published elsewhere.³⁵ The laser Doppler flowmeter (PF4000; Perimed, Stockholm, Sweden) used in this study employs an infrared laser diode (780 nm, 1 mW) coupled to a fiber optic probe (PF403, 0.25 mm fiber separation; Perimed). The flowmeter was calibrated so that the flux registered 250 perfusion units (PU) when the probe was placed in a suspension of latex particles at 22°C, and 0 PU when placed against a plastic disc. The absence of a 0 offset was confirmed at the end of each experiment when the animal was euthanatized.

Ciliary blood flow was measured transsclerally by placing the LDF probe on the scleral surface over the ciliary body (posterior to the limbus and anterior to the low blood flow reading at the pars plana) using a record player tonearm to hold the probe at the same location with the same force throughout the experiment.³⁶ The LDF measurement depth is sufficient to measure through the sclera to the underlying ciliary body. Choroidal blood flow

was measured with a probe advanced through the pars plana with a manipulator so that the probe tip was positioned in the vitreous, near the retinal surface over the posterior pole. For the wavelength (780 nm) and fiber separation (0.25 mm) used in this study, the volume of tissue sampled by the flowmeter is approximately 1 mm³, which is sufficient to measure perfusion in both the retina and the choriocapillaris. As the rabbit retina is mostly avascular and the probe was directed away from the few extant retinal vessels, the flux signal in this preparation originates solely from the choroid.

Protocols

Because of the large size of the fluorophotometer, it was not possible to measure aqueous flow and ciliary blood flow simultaneously. It was also difficult to consistently measure ciliary and choroidal blood flow simultaneously. Consequently, separate groups of animals were used to study aqueous flow ($n = 36$), ciliary blood flow ($n = 26$), and choroidal blood flow ($n = 13$).

In our experience, reliable aqueous flow calculations require measurements of fluorescein clearance for a minimum of 60 minutes, but our rabbit model remained stable for 4 to 5 hours, and so all target MAPs could not be studied in each animal. Thus, in group 1 ($n = 8$), aqueous flow was measured for 60 to 90 minutes before dorzolamide (2%, 50 μ L topical) and for 120 minutes afterward to verify preliminary results indicating that the dorzolamide response was stable after 30 to 45 minutes. In group 2, aqueous flow was measured in separate subgroups that underwent identical 120-minute baseline measurements (MAP, ≈ 70 mm Hg) after application of topical dorzolamide and 1 hour of measurements at one target pressure: 40 mm Hg ($n = 8$), 55 mm Hg ($n = 10$), or 80 mm Hg ($n = 10$). In group 3, ciliary blood flow was measured with the same timing and target pressures as group 2. A significant effort was made to ensure that the measurement conditions were as similar as possible in all animals in groups 2 and 3. In group 4 ($n = 13$), choroidal blood flow was measured during ramp increases and decreases in MAP before and 60 minutes after topical dorzolamide. Representative experiments from protocols 2, 3, and 4 are shown in Figures 1 and 2.

Data Analysis

Aside from the fluorophotometer measurements recorded on a dedicated computer, all variables were recorded with a digital data acquisition system (PowerLab; ADInstruments, Grand Junction, CO) connected to a computer. For group 1, the data were analyzed by averaging all the digitized values during the hour before dorzolamide and during the second hour after dorzolamide. For groups 2 and 3, the data were analyzed by averaging all the digitized values during the hour before and after MAP was set to the target pressure (Fig. 1). For group 4, the aortic and caval occlusions were performed initially after the measured variables were stable for 15 to 30 minutes, then dorzolamide was applied and the occlusions repeated an hour later (Fig. 2). To obtain the choroidal pressure-flow (P-F) curves, we averaged the digitized values for the measured variables during the occlusions in 5-mm Hg bins of perfusion pressure ($PP = MAP - IOP$). Ciliary and choroidal vascular resistances were calculated by dividing the perfusion pressure by the blood flow. Paired and unpaired t -tests were used to assess baseline drug effects within and between groups, respectively (Stat-

View; Abacus Concepts, Berkeley, CA). Repeated measures analysis of variance with two within factors (treatment and PP) followed by paired contrast with the Huynh-Feldt adjustment (SuperANOVA; Abacus Concepts) was used to assess the effect of dorzolamide on the responses of choroidal blood flow to MAP manipulation. Based on a power analysis of results from previous similar studies,^{24,25,36–39} group sizes of 8 to 10 enable detection of <20% changes in all measured variables with a power of 0.8 and an $\alpha = 0.05$. $P < 0.05$ were considered significant. All results are expressed as the mean $\pm$ SE.

As in a previous control study,²⁵ the relationship between ciliary blood flow and aqueous production was estimated by fitting the data from groups 2 and 3 with the following arbitrarily chosen function similar to a Michaelis-Menten equation for enzyme kinetics:

$$AqF = K_1 [CilFlux^{K_2} / (K_3 + CilFlux^{K_2})]$$

where AqF is aqueous flow, CilFlux is ciliary blood flow, and K_1 , K_2 , and K_3 are constants.

Results

Baseline Summary: Groups 1 to 4

Table 1 summarizes the effect of topical dorzolamide on the baseline values for the measured variables in the four groups. A significant increase occurred in ciliary blood flow and significant decreases occurred in IOP, orbital venous pressure, aqueous flow, and ciliary vascular resistance. MAP, choroidal blood flow, and choroidal vascular resistance were unchanged.

Aqueous Flow Protocol: Group 1

Dorzolamide decreased aqueous flow from 3.03 ± 0.17 to $2.50 \pm 0.17 \mu\text{L}/\text{min}$ ($-17.0\% \pm 4.6\%$, $P < 0.01$) and IOP from 14.4 ± 1.0 to $11.8 \pm 0.8 \text{ mm Hg}$ ($-17.4\% \pm 2.0\%$, $P < 0.01$). MAP was unaffected by dorzolamide.

Ciliary Blood Flow and Aqueous Flow Protocols: Groups 2 and 3

Figure 3A shows that the baseline and target MAPs and IOPs were comparable in groups 2 and 3. Systemic parameters such as carotid blood flow and heart rate measured to verify preparation stability were also comparable between groups (data not shown). Figures 3B and 3C show the ciliary blood flow and aqueous flow plotted as a function of perfusion pressure. As intended, changing the perfusion pressure achieved a fairly wide range of ciliary blood flow, although modest autoregulation occurred in the midperfusion pressure range (i.e., ciliary blood flow was not significantly different at perfusion pressures of 58 ± 1 and $46 \pm 1 \text{ mm Hg}$). Aqueous flow was stable, except at the lowest perfusion pressure, when it decreased significantly. Figure 4 shows the relationship between ciliary blood flow and aqueous flow when the data from groups 2 and 3 are combined. The control curve obtained in the same rabbit model using the same protocols and data analysis is shown for comparison.

Choroidal Blood Flow: Group 4

Figure 5 shows that the choroidal pressure-flow relationship before and after topical dorzolamide did not differ significantly when perfusion pressure is varied over a wide range.

Discussion

This study had two goals: to determine the effect of topical dorzolamide on choroidal and ciliary blood flow and to characterize the relationship between ciliary blood flow and aqueous production when production is impaired by interference with ciliary ionic transport.

Ciliary and Choroidal Blood Flow

Acute, topical dorzolamide had no effect on choroidal vascular resistance or blood flow. The choroidal findings are similar to reports by other groups that also found no effect of acute or chronic dorzolamide treatment on posterior ocular blood flow parameters in rabbits and humans.^{9,10,40} One possible reason for the lack of effect on the choroidal vasculature is limited delivery of topical dorzolamide to the back of the eye, although in a study by Pillunat et al.,⁴⁰ no effect on optic nerve head blood flow was found after 3 days of topical dorzolamide treatment. Moreover, Inoue et al.⁴¹ found that the drug reaches the back of the eye in less than 60 minutes after a topical dose is applied in rabbits. However, other studies indicate vasodilatory effects on ocular blood flow in normal patients and those with glaucoma.^{17,18,42–45} Moreover, significant increases in preoptic and retinal P_{O_2} are consistent with increased retinal and optic nerve head blood flow.^{19,20} Thus, while our choroidal results are negative, it is possible that a higher dose or long-term administration might elicit a choroidal vasodilatory response.

In contrast to the choroid, topical dorzolamide caused ciliary vasodilation and increased ciliary blood flow. The diffusion distance from the point of drug application (the cornea) to the ciliary body is much shorter than the distance to the posterior choroid measurement site, and so greater drug delivery may explain the more robust ciliary response. However, dorzolamide's vascular mechanism of action is presumed to be caused by tissue hypercapnia or changes in local pH,^{21,22,46} and the greater amount of metabolically active tissue adjacent to the resistance arterioles in the ciliary body than in the choroid may also contribute to the discrepant vascular effects.

Ciliary Blood Flow and Aqueous Production

A prior study characterizing the relationship between ciliary blood flow and aqueous flow under control conditions found that aqueous flow is unaffected by manipulating ciliary blood flow until blood flow is reduced below a critical point, whereupon aqueous flow declines with further reductions in blood flow.²⁵ As in the present study, ciliary blood flow was manipulated by physically altering arterial pressure, rather than pharmacologically. Based on findings in another secretory tissue,⁴⁷ it was hypothesized that ciliary blood flow delivers oxygen and nutrients needed to sustain ciliary metabolism and the active ionic transport processes responsible for aqueous production. With adequate ciliary blood flow, the rate of aqueous production is set by neurohumoral inputs, and an excess of “fuel”

delivered by increased perfusion would simply pass through with the venous outflow, but insufficient fuel delivery would compromise metabolism and aqueous production.

This hypothesis suggests that decreasing the neurohumoral drive or direct interference with ionic transport in the nonpigmented epithelium would decrease aqueous production to a new set point. This lower rate of aqueous production would still remain insensitive to excess ciliary blood flow and compromised by insufficient ciliary blood flow. It is probable that the critical blood flow rate (below which aqueous production is blood-flow–dependent) would be shifted downward because less fuel is needed to sustain the lower metabolic demand. Such behavior has not been reported previously for aqueous production, but it is similar to what occurs with acid secretion in the gastric mucosa.⁴⁷

This hypothesis was the rationale for the study design for groups 2 and 3. Dorzolamide was chosen because it is a carbonic anhydrase inhibitor thought to decrease aqueous production by limiting the availability of bicarbonate for transport by the nonpigmented epithelium.^{27,28} Figure 4 shows the relationship between ciliary blood flow and aqueous flow obtained under control conditions in the prior study²⁵ and when aqueous production was inhibited with dorzolamide. The results are consistent with the predicted downward shift in the relationship and possibly a leftward shift in the critical point.

However, it is also clear that additional data (e.g., aqueous flows at ciliary blood flows of 30, 40, and 50 PU) would more comprehensively define the relationship under dorzolamide inhibition. Unfortunately, the additional experiments (48 – 60 animals for $n = 8$ –10 per subgroup) were beyond the resources of the laboratory. Additional caveats are that aqueous flow and ciliary blood flow had to be measured in separate groups and that subgroups were needed for the different target arterial pressures. Paired measurements at all target pressures before and after dorzolamide would have been more ideal and would have permitted rigorous statistical comparison. Given these caveats, the relationships shown in Figure 4 should be viewed as current best estimates of the actual relationships.

In summary, acute topical dorzolamide vasodilates the ciliary circulation but fails to alter the posterior choroidal circulation in the rabbit. It also causes an apparent downward shift in the relationship between ciliary blood flow and aqueous flow, consistent with a direct inhibitory mechanism of action. This mechanism is in contrast to those of drugs that decrease aqueous production indirectly by vasoconstricting the ciliary circulation and depriving the ciliary epithelium of the blood flow needed to sustain ciliary metabolism. Distinguishing between direct and indirect mechanisms of action is important for understanding the pharmacology of drugs used to treat glaucoma. However, as with all animal research, these results should be extrapolated to humans or disease states with caution, although it seems likely that the role of blood flow in secretory processes holds across species.

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Figure 1.

Aqueous and ciliary blood flow protocols. **(A)** Representative experimental tracing from the ciliary blood flow protocol (group 3). **(B)** Representative fluorescein concentration decay curves used to calculate aqueous flow (group 2). Data in both graphs are from subgroups in which MAP was lowered to 40 mm Hg. MAP spikes were caused by supplemental anesthetic injections. *Filled symbols*: baseline mean arterial pressure; *open symbols*: at target MAP of 40 mm Hg; *circles*: cornea; *triangles*: anterior chamber. CilFlux, ciliary blood flow.

Figure 2.

Choroidal blood flow protocol. Caval and aortic occlusions were performed before and 1 hour after the application of dorzolamide (2%, topical). *Dashed line*: time of drug application. ChorFlux, choroidal blood flow; R, choroidal vascular resistance.

Figure 3.

(A) MAPs and IOPs at baseline were similar in groups 2 and 3, as were MAPs and IOPs for subgroups at target MAPs. Ciliary blood flow (B) was increased above baseline at the highest perfusion pressure and decreased below baseline at the lowest perfusion pressure. Aqueous flow (C) was altered from baseline only at the lowest perfusion pressure.

Figure 4.

Aqueous flow data (group 2) plotted as a function of ciliary blood flow data (group 3) to approximate the relationship between ciliary blood flow and aqueous production when production is inhibited with dorzolamide. Also shown is the relationship obtained under control conditions in the same model using the same protocols and methods of data analysis.

Figure 5.
Choroidal pressure-flow relationship before (●) and after (○) the application of dorzolamide (2%, topical).

Table 1

Dorzolamide Effects on the Baseline Ocular Parameters Studied

	Control	Dorzolamide	% Change	Group	P
MAP (mm Hg, <i>n</i> = 76)	68.29 ± 0.49	68.51 ± 0.51	0.3 ± 0.50	1+2+3+4	NS
IOP (mm Hg, <i>n</i> = 76)	16.74 ± 0.39	13.39 ± 0.35	-19.4 ± 1.5	1+2+3+4	<0.01
OVP (mm Hg, <i>n</i> = 57)	1.84 ± 0.10	1.69 ± 0.10	-8.8 ± 3.4	1+2+3	<0.01
AqF (μL/min, <i>n</i> = 8)	3.03 ± 0.17	2.50 ± 0.17	-17.0 ± 4.6	1	<0.01
CilFlux (PU, <i>n</i> = 27)	56.20 ± 1.56	65.94 ± 2.20	17.6 ± 2.7	3	<0.01
CilR (mm Hg/PU, <i>n</i> = 27)	0.99 ± 0.03	0.92 ± 0.04	-7.1 ± 2.3	3	<0.01
ChorFlux (PU, <i>n</i> = 13)	825.10 ± 39.12	839.43 ± 42.14	1.6 ± 1.3	4	NS
ChorR (mm Hg/PU, <i>n</i> = 13)	0.063 ± 0.004	0.065 ± 0.004	5.1 ± 3.8	4	NS