Measurement of Aqueous Humor Flow by Fluorophotometry in the Presence of a Dilated Pupil

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Purpose. To determine by means of fluorophotometry whether pharmacologic dilation of the pupil can interfere with the measurement of aqueous flow.

Methods. Ten normal human volunteers underwent dilation with tropicamide, phenylephrine, and a combination of the two drugs. Before and after dilation, the rate of aqueous flow was measured by the rate of disappearance of fluorescein from the cornea and the anterior chamber.

Results. Dilation of the pupil with tropicamide alone had no effect on the rate of clearance of fluorescein. Dilation with phenylephrine increased the rate of clearance of fluorescein by 40% and caused a small increase in the variability among subjects. Dilation with a combination of tropicamide and phenylephrine caused clearance of fluorescein at more than double the normal rate and a marked increase in variability among subjects.

Conclusions. When the pupil is dilated sufficiently to permit mixing of aqueous humor in the posterior and anterior chambers, fluorescein can leave the system by a posterior route, and its rate of clearance may not be an accurate measure of the net rate of aqueous humor flow through the anterior chamber. (*Invest Ophthalmol Vis Sci.* 1999; 40:542-546)

Jones and Maurice[1] introduced a method of measuring aqueous humor flow through the anterior chamber by observing the rate of clearance of fluorescein from the cornea and anterior chamber after topical administration. The method depends on the assumption that all but a small diffusional loss of fluorescein is cleared by the outflow of aqueous as it leaves through the conventional and uveoscleral outflow routes at the iridocorneal angle. This assumption is valid when the iris and crystalline lens form a barrier that prevents mixing of aqueous humor in the anterior chamber with fluids deeper within the eye.

It has been shown that a major disruption of the normal anatomy by removal of the crystalline lens is associated with rapid rates of loss of fluorescein from the eye. Calculations of aqueous flow in some eyes reported in Herman et al.[2] are more than five times normal. It is less clear, however, whether dilation of the pupil is sufficient to disrupt the normal iridolenticular barrier to posterior mixing of aqueous humor. In one study, administration of 0.4% tropicamide caused a 15% decrease in the calculated rate of aqueous humor flow.[3] Administration of 2.5% phenylephrine in another study had no effect on the calculated rate of aqueous humor flow[4] but administration of 5% phenylephrine in other studies caused a 121% increase[5] and a 125% increase[6] in the calculated rate of aqueous humor flow 2 hours after administration. Another dilator of the pupil, ibopamine, a catecholamine with D1 and α-adrenergic agonist activity, has been shown to increase the calculated rate of aqueous humor flow in healthy subjects by 175%.[7] The increases in calculated flow in these studies were not accompanied by commensurate increases in intraocular pressure. It is possible that dilation per se causes the more rapid loss of fluorescein, rather than the other pharmacologic effects of these drugs.

This study was carried out to observe the effect of dilation with two pharmacologic agents of different classes: tropicamide, a cholinergic antagonist, and phenylephrine, an α-adrenergic agonist—when used singly and in combination—on the rate of disappearance of topically applied fluorescein. The hypothesis to be tested was that dilation of the pupil to a very large diameter is, in some subjects, associated with a rapid rate of loss of fluorescein from the anterior chamber that is inconsistent with an increase in the rate of aqueous humor production.

Methods

Subjects and Materials

Ten normal human volunteers were studied (mean age, 25 years; 5 women and 5 men). Exclusion criteria were the following: pregnancy or lactation, use of systemic medications, long-term use of eye medications, history of allergy to ocular medications, history of a major systemic illness, history of significant eye disease, and concurrent or recent participation in another study. Subjects were included only if the intraocular pressures were inside the inclusive range of 10 mm Hg to 20 mm Hg; the intraocular pressures of the two eyes differed 3 mm Hg or less; the lids, globes, and pupils were symmetrical, and myopia or hyperopia of less than 5 D was present. The research followed the tenets of the Declaration of Helsinki, informed consent was obtained from the subjects, and the study was approved by the Mayo Institutional Review Board. Commercial supplies of 1% tropicamide hydrochloride (Mydriacyl; Alcon, Humacao, Puerto Rico), 10% phenylephrine hydrochloride (Steris Laboratories, Phoenix, AZ), and an artificial tear preparation (Hypotears; IOLab, Claremont, CA) were used.

Study Design and Sequence

Aqueous flow of both eyes was measured on 3 days (parts I, II, and III), with a minimum of 1 week between each measurement. Each subject was treated with a dilating drop in one eye (chosen at random) and a placebo drop in the fellow eye. Dilation was achieved with 1% tropicamide in part I, 10% phenylephrine in part II, and both drugs in part III.

At 2 AM the night before the measurement, each subject instilled 2% sodium fluorescein (IOLab) into each eye several times to produce a depot of fluorescein in the cornea for

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Supported in part by Grant EY 00634 from the National Institutes of Health, Bethesda, Maryland; an unrestricted grant from Research to Prevent Blindness, New York, New York; and the Mayo Foundation, Rochester, Minnesota.

Submitted for publication March 11, 1998; revised September 11, 1998; accepted October 9, 1998.

Proprietary interest category: N.

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TABLE 1. Estimated Power of the Sample to Detect Differences in Paired Measurements of Parameters

Variable	Mean Value in Placebo Eye	Coefficient of Variation of Repeated Measures (%)	Minimal Detectable Change	Reference
Aqueous flow	2.6 μl/min	16	± 0.47 μl/min (18%)	9
Intraocular pressure	12.4 mm Hg	9	± 1.24 mm Hg (10%)	10
Volume of anterior chamber	248 μl	4	± 12.4 μl (5%)	11
Diameter of the pupil	4.6 mm	2	± 0.11 mm (2.3%)	12

measurement of aqueous humor flow the following morning. The subjects reported to the research area at 8 AM, and fluorescences in the cornea and anterior chamber were measured with a scanning ocular fluorophotometer. Fluorescence measurements were repeated every hour until 1 PM. After the 10 AM scan, the investigator administered the dilating drop(s) in one eye and the placebo drop in the fellow eye. After the 9 AM and 11 AM scans, pupillographic measurements and anterior chamber volume measurements were made (on the first day). After the 1 PM scan, the intraocular pressure was measured with a Goldmann tonometer using fluorescein sodium 0.25%, benoxinate hydrochloride 0.4% (Fluress; Barnes Hind, Sunnyvale, CA). Intraocular pressure was measured three times in each eye, beginning in the right eye and alternating between eyes. The intraocular pressure was recorded as the mean of the three measurements. At least 1 week elapsed between parts I, II, and III to allow the dilated pupil to recover.

Pupillography

Pupil diameter was measured with a binocular infrared pupillometer.[8] Subjects were seated in a room with illuminance of 20 foot-candles. Two infrared cameras measured the horizontal diameter of each pupil to ±0.1 mm.

Statistical Analysis

The effects of tropicamide and phenylephrine on pupil dilation, anterior chamber volume, intraocular pressure, and aqueous flow were tested by a *t*-test for paired samples in two ways: eye to fellow eye measured simultaneously and eye to same eye measured before and after drug administration. $P \leq 0.05$ was regarded as statistically significant. In Table 1 the estimated power of the sample to detect differences between paired measurements of aqueous flow, intraocular pressure, volume of the anterior chamber, and diameter of the pupil ($n = 10$; $\alpha = 0.05$; $\beta = 0.90$; two-sided paired test) is shown.

RESULTS

On the screening examination, intraocular pressure was measured in both eyes. The pressure in the eyes designated placebo eyes was 15.6 ± 1.8 mm Hg and in the eyes designated treated eyes was 15.8 ± 2.0 mm Hg. There was no statistically significant difference between the spontaneous pressures in the two sets of eyes before the experiment was run.

Tropicamide

Tropicamide caused the pupil to dilate to 8.4 ± 1.0 mm (mean ± SD) and caused a small increase in the volume of the anterior chamber (Table 2). Tropicamide caused no change in the calculated rate of flow of aqueous humor or in the intraocular pressure.

TABLE 2. Part I: Tropicamide

Time	Placebo Eye	Treated Eye	% Difference	*P*
Diameter of pupil (mm)				
Before treatment	4.6 ± 0.6	4.5 ± 0.6	−2	0.4
After treatment	3.9 ± 0.4	8.4 ± 1.0	115	<0.001
P	<0.001	<0.001		
Volume of chamber (μl)				
Before treatment	247 ± 32	241 ± 32	−2	0.09
After treatment	242 ± 26	258 ± 26	7	0.02
P	0.3	0.002		
Aqueous flow (μl/min)				
Before treatment	2.47 ± 0.51	2.64 ± 0.69	7	0.3
After treatment	2.42 ± 0.43	2.55 ± 0.55	5	0.5
P	0.8	0.6		
Intraocular pressure (mm Hg)				
After treatment	12.7 ± 3.1	13.2 ± 3.0	4	0.2

Values are means ± SD.

TABLE 3. Part II: Phenylephrine

Time	Placebo Eye	Treated Eye	% Difference	P
Diameter of pupil (mm)				
Before treatment	4.6 ± 0.8	4.6 ± 0.8	0	0.7
After treatment	4.1 ± 0.6	7.0 ± 1.1	71	<0.001
P	0.004	<0.001		
Volume of chamber (μl)				
Before treatment	247 ± 32	241 ± 32	−2	0.09
After treatment	243 ± 34	250 ± 26	3	0.2
P	0.3	0.1		
Aqueous flow (μl/min)				
Before treatment	2.56 ± 0.52	2.58 ± 0.58	1	0.9
After treatment	2.43 ± 0.34	3.40 ± 0.87	40	0.05
P	0.4	0.004		
Intraocular pressure (mm Hg)				
After treatment	12.1 ± 2.3	13.2 ± 2.4	9	0.02

Values are means ± SD.

Phenylephrine

Phenylephrine caused the pupil to dilate to 7.0 ± 1.1 mm but did not cause any change in the volume of the anterior chamber (Table 3). The calculated rate of flow of aqueous humor was 40% greater in the phenylephrine-treated eye than in the placebo-treated eye after treatment ($P = 0.005$). The coefficient of variation of flow from one subject to another in the placebo-treated eye was 14%, whereas in the phenylephrine-treated eye it was 26%. The intraocular pressure was 9% higher in the phenylephrine-treated eye than in the placebo-treated eye after treatment ($P = 0.02$).

Tropicamide–Phenylephrine Combination

The combination of both drugs dilated the pupil to 9.3 ± 0.7 mm and increased the volume of the anterior chamber (Table 4). The calculated rate of aqueous humor flow was 132% greater in the drug-treated eye than in the placebo-treated eye ($P = 0.004$). Also, the flow in the treated eye varied markedly among subjects; the intersubject coefficient of variation in the drug-treated eye was 54%. Four subjects had high calculated rates of aqueous flow after the drug combination. Despite the large difference in the calculated flow between the placebo-treated eye and the drug-treated eye, there was no significant difference in the intraocular pressure of the two groups of eyes ($P = 0.2$). In addition, the four subjects who were observed to have a markedly large increase in calculated flow and notably large pupils after combined treatment did not have corresponding increases of intraocular pressure (Table 5).

Dilation of the pupil was not always accompanied by an increase in the rate of loss of fluorescein, as was seen with tropicamide. However, when the pupil became much enlarged, as with the combination of tropicamide and phenylephrine, some subjects were observed to have a rapid rate of disappearance of fluorescein. The calculated rate of aqueous flow in such cases was high, as in subject 9 who had a calculated rate of flow of 12.5 μl/min with a 10.0-mm pupil. On slit lamp examination, this subject was clearly observed to have separation of the pupillary border of the iris and the anterior surface of the lens, a condition that would permit leakage of fluorescein-stained aqueous humor from the anterior chamber into the posterior chamber.

TABLE 4. Part III: Combined Tropicamide and Phenylephrine

Time	Placebo Eye	Treated Eye	% Difference	P
Diameter of pupil (mm)				
Before treatment	4.5 ± 0.7	4.6 ± 0.7	2	0.6
After treatment	3.8 ± 0.4	9.3 ± 0.7	145	<0.001
P	0.005	<0.001		
Volume of chamber (μl)				
Before treatment	247 ± 32	241 ± 32	−2	0.09
After treatment	242 ± 34	250 ± 26	3	0.08
P	0.3	0.2		
Aqueous flow (μl/min)				
Before treatment	2.98 ± 1.45	3.22 ± 1.77	8	0.2
After treatment	2.86 ± 0.91	6.63 ± 3.56	132	0.004
P	0.7	0.007		
Intraocular pressure (mm Hg)				
After treatment	12.6 ± 2.5	13.1 ± 2.5	4	0.2

Values are means ± SD.

TABLE 5. Summary of Data from Individual Subjects

		Tropicamide				Phenylephrine				Both Drugs			
Subject*	Eye Color	Pupil Diameter, Treated Eye	Δ IOP†	Gap‡	Δ Flow§	Pupil Diameter, Treated Eye	Δ IOP	Gap	Δ Flow	Pupil Diameter, Treated Eye	Δ IOP	Gap	Δ Flow
6	Blue	7.6	−0.3	0	−0.4	8.1	−0.3	0	0.4	9.1	−0.3	0	0.1
10	Blue	9.5	0.0	0	1.1	5.7	2.3	0	1.4	9.2	0.7	0	1.5
1	Blue	8.9	−0.4	0	0.0	7.7	1.0	0	1.3	9.4	1.0	+	1.7
2	Brown	7.8	1.7	0	0.2	5.5	0.0	0	0.1	9.5	1.0	0	1.9
5	Blue	8.8	2.0	0	−0.1	6.4	0.0	0	−0.2	9.5	0.3	+	2.1
8	Hazel	6.8	1.0	0	−0.7	5.6	3.0	0	0.7	7.7	−1.7	0	2.2
7	Blue	7.5	−1.4	0	1.0	7.3	1.0	0	1.6	8.8	−0.3	+	5.7
3	Green	8.2	1.0	0	−0.1	6.8	1.0	0	1.4	9.9	2.0	+	6.3
4	Brown	10.0	1.7	0	0.1	8.3	2.4	0	0.3	10.2	1.3	+	6.6
9	Brown	90	0.0	0	0.1	8.2	0.3	0	2.6	10.0	1.3	++	9.6

* Sorted by increasing flow in eye treated with both drugs (last column).

† The intraocular pressure of the drug-treated eye minus the placebo-treated eye in millimeters of mercury.

‡ Presence of a gap between the pupillary border of the iris and the anterior surface of the crystalline lens on slit lamp examination: 0, no gap visible; +, small gap visible; ++, large gap visible.

§ The calculated rate of aqueous flow in the drug-treated eye minus the placebo-treated eye.

DISCUSSION

Once in the posterior chamber, fluorescein can diffuse into the anterior hyaloid membrane or be taken up by the pars plicata of the ciliary body. The notion that the rapid disappearance of fluorescein in subject 9 and several others was caused by posterior loss and not by increased aqueous humor formation is supported by the finding that the intraocular pressure remained unchanged relative to the placebo-treated eye. An increase in flow without an increase in pressure could have occurred if outflow had been affected in a compensatory way. We have found no evidence that phenylephrine or tropicamide has a stimulatory effect on aqueous humor flow or diminishes outflow resistance, although both drugs are used widely as part of clinical examinations of patients with and without glaucoma. Also, an increase of pressure could have occurred transiently and could have returned to normal by the time pressure was measured. The experiment was designed to optimize the measure of flow; thus repeated pressure measurements needed to detect transient changes were intentionally omitted.

Phenylephrine has been shown by others to cause an increase in the rate of disappearance of fluorescein[5,6] during the first hour or two, followed by a decrease in the rate of disappearance. Van Genderen et al.[5] and Araie et al.[6] also raised the issue of the pupil but were unable to explain their results on the basis of pupillary dilation alone. For example, van Genderen et al.[5] instilled a second dose of phenylephrine when the pupil was already dilated and observed a second dip in cameral fluorescence. Also, tropicamide was not found to increase flow in their study and in the study of Mori et al.[3] similar to our finding. When individual flows were reported, there was considerable variation among subjects after administration of phenylephrine in contrast with the much tighter range of flows before drug administration. Whatever phenylephrine's effect, it varies from one person to another.

One hypothesis about the effect of phenylephrine is that it breaks down the blood-aqueous barrier and causes an increase in the concentration of albumin in the anterior chamber that quenches the fluorescence of fluorescein, because it is known that albumin reduces the fluorescent efficiency of fluorescein.[13] Araie et al.[6] have measured the intensity of backscattering of light from the anterior chamber in people treated with 5% phenylephrine. They observed a decrease in the scattered light, indicating a lower concentration of colloids even though they calculated an increase in the rate of entry of colloids caused by the large increase in the calculated rate of aqueous flow. Because scattering was reduced after phenylephrine, quenching of fluorescence by albumin could not be an explanation for their observations or for ours.

Another explanation for the phenylephrine result is that this drug dilates the pupil without causing cycloplegia. Our subjects were free to use their eyes between measurements, and the changes in accommodation and pupil size could have allowed posterior escape of the tracer. With tropicamide, however, the pupil and the ciliary body are paralyzed and the dilator muscle relaxed in a lighted environment. Whether the phenylephrine result is an artifact or whether phenylephrine has a direct pharmacologic effect on flow cannot be resolved without additional study.

There was no significant change in the photogrammetrically measured volume of the anterior chamber after application of phenylephrine although there was after application tropicamide. However, tropicamide did not increase flow, whereas phenylephrine did. Thus, the results cannot be explained simply by a change in the volume of the anterior chamber.

Slit lamp examination of our subjects at the maximum point of dilation suggests some separation between the iris and lens in subjects in whom flows were the highest. Also, the flow outliers were generally subjects with the largest pupils, suggesting some role for posterior leakage of fluorescein.

It must be kept in mind that our subjects were active between measurements. Changes in illumination and in accommodative effort can have affected the size of the pupil in the eyes treated with phenylephrine, a drug that does not affect

the powerful iris sphincter or the ciliary muscle. Tropicamide by contrast paralyzes the ciliary muscle and the iris sphincter. Whether residual motion of the iris and lens caused some dye to enter the posterior chamber in the phenylephrine-treated eyes of some subjects is not clear.

Whatever the interpretation of these data, it seems prudent to regard fluorescein clearances in eyes with widely dilated pupils with some skepticism. The notion that some fluorescein leaks into the posterior chamber is not far-fetched under these circumstances. The case for skepticism is further encouraged by the absence of corroboration between measurements of flow and measurements of intraocular pressure.

References

1. Jones RE, Maurice DM. New methods of measuring the rate of aqueous flow in man with fluorescein. *Exp Eye Res.* 1966;5:208-220.
2. Herman DC, Palestine AG, Nussenblatt RB. Ocular fluorescein clearance in patients with hypotony secondary to chronic uveitis. *J Ocular Pharmacol.* 1988;4:327-333.
3. Mori M, Araie M, Sakurai M, Oshika T. Effects of pilocarpine and tropicamide on blood-aqueous barrier permeability in man. *Invest Ophthalmol Vis Sci.* 1992;33:416-423.
4. Lee DA, Brubaker RF. Effect of phenylephrine on aqueous humor flow. *Curr Eye Res.* 1982/1983;2:89-92.
5. van Genderen MM, van Best JA, Oosterhuis JA. The immediate effect of phenylephrine on aqueous flow in man. *Invest Ophthalmol Vis Sci.* 1988;29:1469-1473.
6. Araie M, Mori M, Oshika T. Effect of topical phenylephrine on the permeability of the blood-aqueous barrier in man. *Graefes Arch Clin Exp Ophthalmol.* 1992;230:171-174.
7. Virno M, Taverniti L, De Gregorio F, Sedran L, Longo F. Increase in aqueous humor production following D1 receptors activation by means of ibopamine. *Int Ophthalmol.* 1997;20:141-146.
8. McLaren JW, Fjerstad WH, Ness AB, Graham MD, Brubaker RF. New video pupillometer. *Optical Eng.* 1995;34:676-683.
9. Brubaker RF. Clinical measurements of aqueous dynamics. Implications for addressing glaucoma. *Curr Topics Membr.* 1998;45: 233-284.
10. Dielemans I, Vingerling JR, Hofman A, Grobbee DE, de Jong PTVM. Reliability of intraocular pressure measurement with the Goldmann applanation tonometer in epidemiological studies. *Graefes Arch Clin Exp Ophthalmol.* 1994;232:141-144.
11. Johnson S, Coakes RL, Brubaker RF. A simple photogrammetric method of measuring anterior chamber volume. *Am J Ophthalmol.* 1978;85:469-474.
12. McLaren JW, Fjerstad WH, Ness AB, Graham MD, Brubaker RF. New video pupillometer. *Optical Eng.* 1995;34:676-683.
13. Grotte D, Mattox V, Brubaker RF. Fluorescent, physiological and pharmacokinetic properties of fluorescein glucuronide. *Exp Eye Res.* 1985;40:23-33.