Prostaglandin $F_{2\alpha}$-1-Isopropylester Lowers Intraocular Pressure Without Decreasing Aqueous Humor Flow

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Using fluorophotometry, we performed a randomized, dose-response study of the effects of a prostaglandin derivative on aqueous humor flow. Prostaglandin $F_{2\alpha}$-1-isopropylester, 0.224 μg, 0.448 μg, and 1.120 μg, in saline with polysorbate 80 was instilled into one eye of 20 subjects in three separate dose studies. Polysorbate 80 in saline was instilled in the fellow eye as a control. The drug had no measurable effect on aqueous humor flow or corneal endothelial permeability. Intraocular pressure measured eight hours after administration of the highest dose, 1.120 μg, was 20% lower in the treated eye as compared to the fellow eye ($P < .001$).

PROSTAGLANDINS administered topically reduce intraocular pressure in several species.[1] For example, topical administration of prostaglandin $F_{2\alpha}$ (tromethamine salt) reduces intraocular pressure in rabbits.[2] Lee, Podos, and Severin[3] noted reductions in intraocular pressure in rabbits, cats, and monkeys after topically administered prostaglandin $F_{2\alpha}$ (tromethamine salt) in doses ranging from 250 to 1,000 μg. They observed no changes in aqueous humor flow as measured by fluorophotometry, but did observe significant increases in outflow facility as measured by tonography. Additional studies in cats and rhesus monkeys have shown that topically applied prostaglandin $F_{2\alpha}$ (tromethamine salt) produces a transient (15- to 30-minute) increase in intraocular pressure,[4] followed by a persistent decrease in intraocular pressure without development of tachyphylaxis when twice-daily doses are given.[5]

A study of owl monkey eyes showed that 1,000-μg doses of prostaglandin $F_{2\alpha}$ (tromethamine salt) can reduce intraocular pressure by 4.7 mm Hg in normal eyes and 25 mm Hg in glaucomatous eyes.[6] Crawford, Gabelt, and Kaufman[7] showed that 100-μg doses of topically administered prostaglandin $F_{2\alpha}$ (form not specified) produced an 8-mm Hg reduction in intraocular pressure and a reduction in aqueous humor flow in some but not all of their experiments using cynomolgus monkeys.[7] Camras and associates[8,9] noted a 10-mm Hg reduction of intraocular pressure in the same species without an accompanying reduction of aqueous humor flow after a 250-μg topical dose of prostaglandin $F_{2\alpha}$ (tromethamine salt). These investigators attributed the effect to changes of outflow facility.

In a study of 18 nonglaucomatous subjects, Giuffre[10] administered a 200-μg topical dose of prostaglandin $F_{2\alpha}$ (tromethamine salt) and noted significant reductions of intraocular pressure in treated eyes as compared to control eyes. The studies cited here have shown consistent reductions of intraocular pressure in several species with high doses of prostaglandin $F_{2\alpha}$.

Bito and Barody[11] have shown that the corneal epithelium acts as a barrier to prostaglandin $F_{2\alpha}$. Bito[12] has subsequently studied several esters of prostaglandin $F_{2\alpha}$ and found that methyl, ethyl, and isopropyl esters reduce intraocular pressure in cats at much lower doses than prostaglandin $F_{2\alpha}$. He also observed that the isopropyl ester of prostaglandin $F_{2\alpha}$ provided longer-term effects than the methyl or ethyl esters.

In a study of 0.1-, 0.5-, and 10-μg doses of prostaglandin $F_{2\alpha}$-1-isopropylester in six normal male volunteers, a dose-related reduction of intraocular pressure with a maximum reduc-

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tion eight hours after instillation was noted (A. Alm, M.D., and J. Villumsen, M.D., personal communication, 1985). The mean reduction of intraocular pressure eight hours after instillation was 5.7 mm Hg ($P < .02$). Villumsen and Alm[13] also studied 0.5-μg doses of prostaglandin $F_{2\alpha}$-1-isopropylester administered at 8 A.M. and 8 P.M. for one day to 12 glaucomatous patients. They observed intraocular pressure at eight, 12, and 24 hours after the first dose and noted mean reductions in pressure of 5.7 mm Hg ($P < .001$), 7.9 mm Hg ($P < .001$), and 7.3 mm Hg ($P < .005$), respectively.

The purpose of this study was to measure the rate of flow of aqueous humor in the human eye after the topical administration of a range of doses of prostaglandin $F_{2\alpha}$-1-isopropylester known to lower intraocular pressure.

Subjects and Methods

We selected 20 volunteers (11 men and nine women), ranging in age from 21 to 60 years, with no history of eye disease or significant systemic disease for the study. Subjects were excluded from the study for any of the following: (1) active ocular disease, (2) anatomic differences in the size and color of their eyes, (3) systemic drug use, (4) inability to undergo satisfactorily tonometry or fluorophotometry, (5) suspected hypersensitivity to drugs, or (6) participation in a trial of any other investigational drug within two weeks. The screening examination included a medical and ophthalmic history, visual acuity testing, slit-lamp examination, Goldmann applanation tonometry, and direct ophthalmoscopy. Written, informed consent according to federal guidelines was obtained from all subjects. Preliminary tests included photogrammetry to measure the volume of the anterior chamber.[14] Autofluorescence of each cornea was also measured.[15] Results of blood tests for pregnancy were negative two to three days before beginning the study in all female subjects.

Prostaglandin $F_{2\alpha}$-1-isopropylester was used as an ophthalmic solution at a concentration of 22.4 μg/100 ml. The drug was instilled by micropipette in 1-, 2-, and 5-μl doses, which contained 0.224, 0.448, and 1.12 μg of the investigational drug, respectively. The study was conducted under investigational new drug exemption No. 28,391 of the Food and Drug Administration.

Ten left and ten right eyes were randomly assigned to the study group. Each subject received a single 1-, 2-, and 5-μl dose of the test drug in the selected eye and a placebo (polysorbate 80 in saline) in the fellow eye in three separate studies spaced by a minimum of two weeks.

At 2 A.M. on the day of the study each subject instilled 2% fluorescein into each eye every three minutes for a total of five to ten instillations per eye. The number of instillations was individualized for each subject to produce the optimal concentration of fluorescein in the stroma.

Subjects reported to the testing area at 8 A.M. on the day of the study. Intensity and polarization of fluorescein were measured in both corneas and anterior chambers using the two-dimensional scanning fluorophotometer of McLaren and Brubaker.[16] After these measurements, the drug and placebo were instilled into the lower cul-de-sac of the subjects by micropipette; a total volume of 1, 2, or 5 μl was delivered containing 0.224 μg, 0.448 μg, and 1.120 μg of prostaglandin $F_{2\alpha}$-1-isopropylester, respectively. The subjects were instructed to keep their eyelids closed for four minutes after instillation to retard washout of the drug by tears. Hourly measurements of fluorescein in the cornea and anterior chamber were then made on all subjects.

Polarization of fluorescence was measured in each eye four to eight hours after instillation of the drug. At 4 P.M. on the day of the study (eight hours after drug instillation), intraocular pressure was measured in each eye by Goldmann applanation tonometry by one of us (S.E.W.) who did not know which eye had been treated. The measurements were taken three times, beginning with the right eye and alternating between eyes, and then averaged. Pretreatment intraocular pressures were measured in the same manner during the screening examination. Pretreatment intraocular pressures were not measured on the study day because of the confounding effect that fluorescein-containing topical anesthetics would have had on the measurement of aqueous humor flow by fluorophotometry. At the conclusion of the study, an ophthalmic examination was again performed. Each subject was queried about symptoms, and any symptoms reported were recorded.

Aqueous humor flow was calculated by a modification[17] of method 2 of Jones and Maurice.[18] The rate of clearance of fluorescein was

calculated as the rate of disappearance of fluorescein from the combined cornea and anterior chamber divided by the average fluorescent intensity in the anterior chamber during that interval. The rate of diffusional clearance of fluorescein was assumed to be 0.25 μl/minute.[19]

Results

All 20 subjects completed the study of the 1- and 2-μl doses. However, two subjects complained of pain and photophobia and were noted to have marked conjunctival hyperemia for several hours after instillation of the 2-μl dose. They were excluded from tests of the 5-μl dose.

Neither of the two smaller doses caused a significant reduction of intraocular pressure eight hours after instillation (Table 1). The highest dose produced an ocular hypotensive effect that was significant eight hours after instillation. Intraocular pressure was 11.8 ± 2.8 mm Hg in the treated eye and 14.4 ± 3.0 mm Hg in the untreated eye, a difference of approximately 20% ($P < .001$).

We were unable to demonstrate any effect of the drug on the rate of aqueous humor flow. The flow was in the range of 2.5 to 2.9 μl/minute under all conditions of the experiment. The rates of flow were generally higher in the morning than the afternoon in both the treated eye and the fellow eye (Table 2), a difference that can be attributed to the diurnal variation of aqueous humor flow in humans.

The permeability of the corneal endothelium to fluorescein was not significantly altered by prostaglandin $F_{2\alpha}$-1-isopropylester (Table 3), nor was there any evidence from polarization of fluorescence in the cornea or anterior cham-

TABLE 1
EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$-1-ISOPROPYLESTER ON INTRAOCULAR PRESSURE

	INTRAOCULAR PRESSURE (MM HG)			
	PRETREATMENT		8 HOURS POSTTREATMENT	
DOSE	MEAN	S.D.	MEAN	S.D.
1 μl (0.224 μg)				
Drug	14.7	2.6	12.9	2.6
Placebo	14.1	2.3	14.0	2.9
	N = 19		N = 20	
2 μl (0.448 μg)				
Drug	14.4	2.4	13.6	3.6
Placebo	14.4	2.5	13.4	2.7
	N = 19		N = 20	
5 μl (1.12 μg)				
Drug	14.4	2.3	11.8*	2.8
Placebo	14.6	2.7	14.4*	3.0
	N = 17		N = 18	

*Difference between drug-treated and placebo-treated eyes was significant ($P < .001$) using two-tailed *t*-test.

TABLE 2
EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$-1-ISOPROPYLESTER ON AQUEOUS HUMOR FLOW (μL/MIN)*

	MORNING (8 A.M.–NOON)		AFTERNOON (NOON–8 P.M.)		AVERAGE (8 A.M.–8 P.M.)	
DOSE	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
1 μl (N = 20)						
Drug	2.72	0.72	2.65	0.47	2.69	0.60
Placebo	2.86	0.77	2.66	0.50	2.76	0.64
2 μl (N = 20)						
Drug	2.88	0.45	2.67	0.49	2.78	0.47
Placebo	2.78	0.62	2.65	0.47	2.72	0.55
5 μl (N = 18)						
Drug	2.67	0.48	2.68	0.53	2.68	0.51
Placebo	2.52	0.47	2.50	0.40	2.51	0.44

*Differences between drug-treated and placebo-treated eyes were not significant.

TABLE 3
EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$-1-ISOPROPYLESTER ON ENDOTHELIAL PERMEABILITY (CM/MIN × 10^{-4})*

	MORNING (8 A.M.–NOON)		AFTERNOON (NOON–8 P.M.)		AVERAGE (8 A.M.–8 P.M.)	
DOSE	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
1 μl (N = 20)						
Drug	3.56	0.96	4.00	0.72	3.78	0.84
Placebo	3.69	0.68	3.94	0.63	3.82	0.66
2 μl (N = 20)						
Drug	3.79	0.63	3.66	0.58	3.73	0.61
Placebo	3.70	0.70	3.65	0.46	3.68	0.58
5 μl (N = 18)						
Drug	3.57	0.58	3.99	0.48	3.78	0.53
Placebo	3.52	0.62	3.91	0.66	3.72	0.64

*Differences between drug-tested and placebo-treated eyes were not significant.

TABLE 4

EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$-1-ISOPROPYLESTER ON POLARIZATION OF FLUORESCENCE*

	PRETREATMENT				POSTTREATMENT			
	CORNEA		ANTERIOR CHAMBER		CORNEA		ANTERIOR CHAMBER	
DOSE	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
1 μl (N = 20)								
Drug	0.167	0.036	0.031	0.023	0.176	0.043	0.033	0.018
Placebo	0.165	0.030	0.025	0.016	0.174	0.025	0.027	0.012
2 μl (N = 20)								
Drug	0.154	0.022	0.023	0.016	0.175	0.023	0.019	0.011
Placebo	0.157	0.023	0.027	0.020	0.184	0.038	0.023	0.013
5 μl (N = 18)								
Drug	0.171	0.023	0.026	0.014	0.188	0.022	0.026	0.015
Placebo	0.172	0.026	0.028	0.017	0.189	0.021	0.026	0.012

*Differences between drug-treated and placebo-treated eyes were not significant.

ber that the drug had caused a breakdown of the blood-aqueous barrier (Table 4). In this experiment, polarization of fluorescence is used to measure any changes in the polarization characteristics of the cornea or anterior chamber that would occur in response to the presence of additional proteins from breakdown in the blood-aqueous barrier.

Two subjects had moderate redness for several hours and ocular discomfort after the 2-μl dose. Otherwise, the drug was well tolerated. All subjects had conjunctival hyperemia that lasted one to several hours after instillation.

Discussion

This study confirms the observations of others[11] that low doses of topically applied prostaglandin esters can reduce intraocular pressure. This pressure lowering effect must be the result of effects on outflow since we did not observe any decrease in the rate of clearance of fluorescein. Since no significant changes in the rate of aqueous humor flow were noted, we calculated the statistical power of the experiment based on a previous study of 71 normal, untreated subjects, in whom the variance of our measurement technique was determined. In the present experiment where n = 20, the probability of detecting an 18% change in flow is 95%. There is a 50% chance that we would have detected changes in flow of approximately 10%. We believe, as did Camras and associates[8,9] and Lee, Podos, and Severin,[3] that prostaglandin $F_{2\alpha}$ has no clinically significant effect on aqueous humor flow and that its ocular pressure lowering effect must be mediated via the outflow system.

Compared to the four major classes of therapeutic ocular hypotensive agents currently used for the treatment of glaucoma—cholinergics, adrenergic agonists, adrenergic blockers, and carbonic anhydrase inhibitors—prostaglandin $F_{2\alpha}$ appears to have effects that are most similar to adrenergic agonists, such as epinephrine. First, epinephrine's effect may be the result of endogenous prostaglandin release.[20,21] Second, both epinephrine[22] and prostaglandin $F_{2\alpha}$[7] are thought to enhance uveoscleral outflow. However, ocular hypotensive response to prostaglandins may also be the result of unique mechanisms not shared by any currently used therapeutic agents.

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OPHTHALMIC MINIATURE

One case of acute and two cases of chronic glaucoma were treated by excisions of a portion of the iris in each eye. The acute case completely recovered with capillary hemorrhage; the two chronic cases are improving.

In cases in which the operation has been successfully performed, the aqueous humour is not accumulated in the anterior chamber, but appears to escape through the wound as it is secreted. No active inflammatory symptoms have occurred after treatment as one would expect after so severe an operation.

British Medical Journal, March 6, 1858, p. 181