Topical application of Prostaglandin F\textsubscript{2α} (PGF\textsubscript{2α}) to the eye reduces intraocular pressure (IOP) in all mammalian species studied thus far, including humans. The L-isopropylester derivative is currently the one most commonly used in experimental and clinical studies. Dose–response relationships were determined between PGF\textsubscript{2α}-IE and IOP, pupillary diameter, and refraction in ketamine-anesthetized ocular normotensive cynomolgus monkeys. Single doses of 10 and 30 µg had smaller and less consistent but longer lasting IOP-lowering effects than repeated doses (twice daily for 3 days) of 1–5 µg. For repeated dosing in this manner, the just-maximal dose is probably between 2–5 µg, producing a ~70% reduction in IOP to a final IOP of ~5 mm Hg. Continuing treatment for up to 18 days did not further enhance the efficacy of twice daily treatment with a submaximal 1-µg dose. Partial reversal of anesthesia-induced tonic accommodation occurred with single 10- and 30-µg doses and with repeated 1-µg doses, but additional myopia of 0.5–1.5 diopters was induced with repeated higher doses. These physiologic findings and previous morphologic data are consistent with a proposed dual PG action on the ciliary muscle, one involving a short-onset long-lasting direct effect on the muscle fibers (causing relaxation and narrowing of the muscle bundles) and the second involving a slowly developing but shorter duration dissolution of the intermuscular connective tissues. Invest Ophthalmol Vis Sci 32:510-519, 1991

Prostaglandin F\textsubscript{2α} (PGF\textsubscript{2α}) is a powerful ocular hypotensive agent in rabbits,\textsuperscript{1,2} cats,\textsuperscript{3-5} dogs,\textsuperscript{2} monkeys,\textsuperscript{3,4,6-8} and humans.\textsuperscript{9-11} However, in contrast to cats and primates, the intraocular pressure (IOP) reduction in rabbits is lost with repeated doses, and, with the exception of the very lowest doses, is associated with a marked breakdown of the blood–aqueous barrier. The isopropylester (IE) derivative of PGF\textsubscript{2α} has replaced the tromethamine salt in experimental studies because of better corneal penetration, but few data regarding the dose–response relationships with this derivative have been reported. A major question with regard to the potential use of PGs as clinical antiglaucoma drugs is separation of the “therapeutic” ocular hypotensive action from “adverse” actions such as miosis, external ocular vasodilation and irritation, and blood–aqueous barrier breakdown. PGF\textsubscript{2α}’s ocular hypotensive effect in primates is due primarily to enhancement of posterior drainage of aqueous humor through the ciliary muscle (uveoscleral outflow).\textsuperscript{12,13} PGs can cause either contraction or relaxation of various smooth muscles,\textsuperscript{14} and may facilitate cholinergic neurotransmission and potentiate the effect of cholinergic agonists.\textsuperscript{15,16} The shape and state of contraction of the parasympathetically dominated ciliary smooth muscle controls accommodation.\textsuperscript{17} Additionally, PGF\textsubscript{2α} tromethamine salt had greater ocular hypotensive efficacy at multiple low doses than at single low or high doses.\textsuperscript{8} Therefore the single and multiple dose–response relationships for PGF\textsubscript{2α}-IE effects on IOP, pupil diameter, and refraction were investigated in the cynomolgus monkey. This species is the subhuman primate most frequently employed to study aqueous humor dynamics and was used to elucidate the mechanism of PGF\textsubscript{2α}’s IOP-lowering effect.

Materials and Methods

The PGF\textsubscript{2α}-IE solutions (0.002–0.3%) were administered topically to one eye of young adult female cynomolgus monkeys (Macaca fascicularis) either as
a single dose or in a multiple-dose regimen. Each dose group contained three to seven animals. The single dose (1, 10, or 30 μg) was administered to ketamine-anesthetized (5–10 mg/kg intramuscularly) monkeys as one 10-μl or two 5-μl drops. Repeated doses (0.2, 0.5, 1.0, 2.0, 2.5, 3.0, or 5.0 μg per treatment) were administered to manually restrained conscious monkeys as two 5-μl drops twice daily (~7:30 AM and ~3:00 PM) for 3 days. On the morning of day 4, the animals were anesthetized with ketamine, and measurements were made before (except for the 0.2 μg dose) and after a seventh PG dose. The IOP, refraction, and pupil diameter were measured (by minified Goldmann tonometer, Hartinger coincidence refractometer, and Vernier calipers [under normal room light], respectively) at 30, 60, 90, and 120 min, and then hourly for 6 or more hr after PG administration. All but two animals treated with multiple doses of PGF2α-IE were examined by slit-lamp biomicroscopy on day 4 of treatment, 2–6 hr after the seventh PG dose.

From the results of the 4-day studies, an effective but submaximal IOP-lowering dose (1.0 μg) was chosen for a longer treatment protocol. For this experiment, five animals were treated twice daily in one eye for 18 days, with IOP, pupil, refraction, and slit-lamp examination on days 4, 11, and 18.

A total of 31 animals were used. Six of the animals were entered in more than one dose-duration protocol, but no animals were entered in more than three. At least 1 week elapsed between protocols for all animals. For each experiment, treated and control eyes were selected randomly. The untreated contralateral eye served as a control for each animal. All experiments were conducted in accordance with the ARVO Resolution on the Use of Animals in Research.

Results

IOP

Single doses of 1, 10, and 30 but not 0.2 μg of PGF2α-IE produced an ipsilateral 3–4 mm Hg rise in IOP at 30 min (Fig. 1). Thereafter, ipsilateral IOP began a prolonged decline whose magnitude and duration were dose dependent. Compared with pretreatment baseline and contralateral control, the hypotensive effect of the 10- and 30-μg doses lasted at least 24 hr and reached a maximum magnitude of ~5 mm Hg, with the full effect occurring more rapidly at the 30-μg dose. However, this hypotensive trend was too variable to be convincingly statistically significant with the number of animals studied.

Twice daily administration of 0.2–5.0 μg of PGF2α-IE (Fig. 2) had little ocular hypertensive effect after the seventh dose on day 4; only at the 5-μg dose was such a tendency seen. Rather, a distinct IOP fall was apparent; the magnitude, duration, and consistency of which were all dose dependent. A maximum IOP reduction of 68 ± 5.3% (mean ± the standard error of the mean, n = 6 animals) from the initial IOP of 13.3 ± 0.7 mm Hg on day 0 to final IOP of 4.2 ± 0.6 mm Hg 6 hr after the seventh 5-μg dose on day 4, was obtained. This consistent 9-mm Hg decrease was approximately twice the variable 5-mm Hg de-
Fig. 2. IOP before PG treatment (day 0) and before and after the 7th dose of PGF$_{2\alpha}$-IE on the 4th day of unilateral treatment. Time 0 occurs ~17 hr after the 6th PG dose (given at ~3:00 PM on day 3) and immediately before the 7th dose (given at ~8:30 AM on day 4). Data in left panels are mean ± SEM IOP for n animals, each contributing one treated and one untreated eye; (●) PGF$_{2\alpha}$-treated eyes; (O) untreated contralateral eyes. Data in right panels are mean ± SEM difference between treated and control eyes. Symbols on abscissa indicate significant difference between treated and control eyes by the two-tailed two-sample (left) or paired (right) t-test. *$P < 0.05$, †$P < 0.02$, ‡$P < 0.01$, §$P < 0.001$. 

Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933161/ on 06/24/2017
crease obtained with the 10- and 30-μg single doses in animals with initial IOPs 1–2 mm Hg higher. The greater efficacy of repeated versus single dosing was also evidenced by the greater magnitude and duration of IOP lowering after repeated (Figs. 2E–F) compared with single (Figs. 1C–D) 1-μg doses. However, the effect of the repeated doses may have been of shorter duration than that of the higher single doses. Seventeen hours after the sixth 2–5-μg dose (ie, just before the seventh dose at time 0 on day 4), IOP was at most only 2–3 mm Hg lower in the treated than in the control eyes (Figs. 2G–N) compared with the 4–5 mm Hg reduction, albeit variable, 24 hr after the single 10- and 30-μg doses (Figs. 1E–H).

The IOP response to twice daily administration of an effective, but submaximal, ocular hypotensive dose (1 μg) was assessed on day 4, 11, and 18 in a subset of five animals. The small IOP reduction 17 hr after the previous PM dose and the magnitude and time course of the IOP fall after the AM dose were essentially the same on all three occasions (Figs. 3A–D). Figure 4 shows the PGF2α dose–IOP response curve representing the maximum IOP-lowering effect after the seventh dose on day 4 of twice-daily treatment.

**Pupillary Diameter**

The three highest single doses tended to produce slight miosis relative to the contralateral controls (data not shown). Miosis of 0.2–0.8 mm relative to the contralateral control was also often present after the seventh dose on treatment day 4 (Fig. 5). Although a dose–response relationship was not clearcut, the effect was of the greatest magnitude, duration, and consistency at the two highest doses (3 and 5 μg).

In the subset of animals treated twice daily with 1 μg for 18 days, the magnitude and duration of the relative miosis was greater on days 11 and 18 than on day 4 (Figs. 3E–H). However, there was no further enhancement of the effect from days 11–18. The effect of 1 μg on days 11 and 18 (Figs. 3F–H) was of similar maximum magnitude (~0.8 mm) but greater duration than the effects of 3 and 5 μg on day 4 (Figs. 5K–N).

**Refractive Error**

Resting refractive error in our untreated ketamine-anesthetized monkeys ranged generally from ~1 to ~4 diopeters (Figs. 6, 7). After single 10- and 30-μg PGF2α-IE doses and after the seventh 1-μg dose on treatment day 4, the treated eye tended to become less myopic than the contralateral control. This effect developed within 1 hr, reached its maximum of ~0.9 diopter in 3–4 hr, and largely dissipated by 6 hr (Figs. 7E–F). The effect was not of sufficient magnitude to render the eyes emmetropic. In the subset of animals treated twice daily with 1 μg for 18 days, the reversal of the resting myopia tended to be of less magnitude and duration on days 11 and 18 than on day 4 (Figs. 3I–L).

In contrast to the 1-μg dose, repeated 2–5-μg doses tended to make the treated eyes slightly more myopic than their contralateral controls on treatment day 4 (Figs. 7G–N). The effect was ~0.5–1.5 diptors and tended to be larger, more consistent, and of longer duration at the two highest doses (3 and 5 μg). Treatment courses longer than 4 days were not studied for doses higher than 1 μg.

**Slit-Lamp Biomicroscopy**

Cells were observed biomicroscopically in the anterior chamber of two PGF2α-IE-treated eyes that received the 2-μg dose for 4 days; no other treated eyes and no control eyes exhibited this finding, and no other abnormality was seen in any eye.

**Discussion**

Our data confirm the potent ocular hypotensive action of PGF2α-IE in the cynomolgus monkey and indicate that the duration and magnitude of the response are both dose dependent. At the largest repeated dose, 5 μg, there was little interanimal variability in the IOP of the treated eye between 1.5–6 hr after the seventh drug dose. The IOP became lower than any rational value for episcleral venous pressure. This is consistent with previous findings that PGF2α's ocular hypotensive effect is primarily due to increased uveoscleral outflow, since the uveoscleral route drains against an intraorbital pressure of ~0 mm Hg. The greater efficacy of repeated smaller doses compared with single larger ones has been noted previously and is consistent with the cell-mediated enzymatic lysis of connective tissue between the ciliary muscle bundles described by Tamm and associates. We would expect such an effect to require some time to become fully manifest.

Although the much larger single doses were less efficacious, their effect may have been longer lasting. This suggests the possibility of another mode of action, whether by affecting uveoscleral outflow (eg, by relaxation of the ciliary muscle without collagenolysis) or an entirely different parameter of aqueous humor dynamics (eg, aqueous formation, trabecular outflow facility, or episcleral venous pressure).

Repeated effective but submaximal ocular hypotensive doses of PGF2α-IE have little if any effect on trabecular outflow facility in the cynomolgus mon-
key. The effect of single PGF2α doses on the trabecular facility has not been studied in any species. Single and repeated doses of PGF2α-IE and PGF2α-tromethamine salt have been reported to increase the total facility measured by tonography or perfusion in the rabbit, cat, cynomolgus monkey, and human. However, we cannot tell which component of the total facility (trabecular, uveoscleral, or inflow) is involved, and in any event, an effect on the total facility is sometimes lacking even in the presence of a significant PGF2α-induced IOP decrease.

Neither single nor repeated doses of PGF2α decrease aqueous formation measured by noninvasive fluorophotometry (cynomolgus monkey, cat, cynomolgus monkey, and human) or invasive isotope dilution techniques (cynomolgus monkey), although the dosages studied were not comparable with our 10- and 30-μg PGF2α-IE doses. Single 1-μg doses of PGF2α-IE may increase aqueous flow slightly in both monkey and human eyes, perhaps contributing to the early IOP rise.

Single ocular-hypotensive 2.5-μg doses of PGF2α-IE do not alter episcleral venous pressure in the cat, but the collector channels in the cat drain into a different venous bed. No data are available regarding PGF2α's effect on episcleral venous pressure in the monkey or human.

Camras et al found no morphological differences between treated and control eyes of cynomolgus monkeys treated unilaterally for 2 weeks with 250 μg PGF2α-tromethamine salt. However, in more detailed studies, Lutjen-Drecoll and Tamm et al reported that repeated 4- and 5-μg doses of PGF2α-IE produce narrowing of the ciliary muscle bundles and widening of the intermuscular spaces in the cynomolgus monkeys, and may also cause lysis and loss of intermuscular connective tissue. The latter alone might increase uveoscleral outflow to a lesser degree than dissolution of the connective tissue. Unlike the connective tissue changes, a direct muscular effect of the prostanoid could develop rapidly. Uveoscleral outflow is increased by ~60% in cynomolgus within the first few hours after a single 1-μg PGF2α-IE dose, but the effects of larger single doses on uveoscleral outflow and ciliary muscle morphology have not been studied. Repeated dosing with PGF2α for 3–4 days has shown to increase uveoscleral outflow 2–4 fold in cynomolgus monkeys.

Thus, the less pronounced but longer lasting IOP fall after large single doses of PGF2α, compared with the larger magnitude but shorter duration decrease after repeated smaller doses could be due to two distinct uveoscleral outflow-increasing effects of the prostanoid. One would be consequent to a relatively rapid-onset direct effect on the ciliary muscle fibers, the second to a more slowly developing enzymatically mediated dissolution of intermuscular connective tissue. However, other explanations, not necessarily limited to those discussed in these paragraphs, are certainly possible.

Whatever the mechanisms for the overall ocular hypotensive effect of repeated dosing, we sought to determine whether the effect had fully developed by day 4 or whether continuing treatment would enhance it further. We chose an effective but clearly submaximal IOP-lowering dose of 1 μg for the longer protocol, since it would have been difficult to detect enhancement at higher doses given the extremely low IOP they had produced by day 4. The magnitude and time course of IOP lowering were essentially the same.
Fig. 5. Pupillary diameter before PG treatment (day 0) and before and after the 7th dose of PGF$_{2\alpha}$-IE on the 4th day of twice-daily unilateral treatment. Time 0 occurs ~17 hr after the 6th PG dose (given at ~3:00 PM on day 3) and immediately before the 7th dose (given at ~8:30 AM on day 4). Data in left panels are mean ± SEM pupil diameter for n animals, each contributing one treated and one untreated eye; (●) PGF$_{2\alpha}$-treated eyes, (○) untreated contralateral eyes. Data in right panels are mean ± SEM difference between treated and control eyes. Symbols on abscissa indicate significant difference between treated and control eyes by the two-tailed two-sample (left) or paired (right) t-test. *P < 0.05; †P < 0.02; ‡P < 0.01.
on treatment days 4, 11, and 18. Assuming that ciliary muscle and connective tissue remodeling are primarily responsible for the ocular hypotension, this indicates that the system is fully primed within 3 days so that each subsequent dose triggers a major (but reversible within hours) response and that priming the system does not itself dramatically reduce IOP.

Our protocol cannot distinguish whether the repeated dose-IOP response relationship occurs as a result of priming or triggering of the system or both, but the findings again suggest that two separate mechanisms may be involved. One could imagine, for instance, that dissolution of the intermuscular connective tissues or PG-induced narrowing of the muscle bundles might each increase uveoscleral outflow and lower IOP only slightly, but that narrowing of the muscle bundles and widening of the connective tissue spaces in the presence of ratified connective tissue could have a far more dramatic effect. A muscular effect of relatively rapid onset and short duration after each dose (including the first) and a connective tissue effect of slow onset and long duration (days) would explain all the findings with single and repeated doses and is physiologically reasonable.

Although proof of this hypothesis clearly requires further experimental work, the data for refractive error are consistent with it. The pretreatment resting myopia of 1-4 diopters in our ketamine-anaesthetized monkeys most probably represents parasympathetically stimulated tonic accommodation. The tendency toward partial reversal of the resting myopia for a few hours after single 10- and 30-μg doses and the seventh 1-μg dose on treatment day 4 could represent muscular relaxation as evidenced by narrowing of the muscle bundles. The diminution of myopia reversal on days 11 and 18 of continuing 1-μg treatments and the mild additional myopia on day 4 of treatment with higher doses could be consequent to the connective tissue effect. If extracellular material between the ciliary muscle bundles is lost, this "glue," which holds the bundles together, may weaken. The normal elasticity of the lens could then be more effective in pulling the muscle inward. Such a scenario could cause both myopia and widening of intermuscular spaces. It would be of interest to attempt to correlate IOP and refractive effects in individual animals at specific time points for the different doses, but such an analysis is beyond the scope of this paper. Whatever the mechanisms, the various PG effects on refractive error are probably too small to be of major clinical significance. In clinical studies on conscious humans not accommodating tonically, neither single PGF2α-IE doses of 0.5, 1, 5, and 10 μg, nor twice daily doses of 0.25 and 0.5 μg for 1 week produced any apparent refractive effects. The PGF2α had only a slight miotic action in our monkeys, much less than that observed in the cat and in the beagle dog. Although the magnitude and duration of the miosis were dose dependent, the miotic effect even after 1 μg twice daily for 18 days or 5 μg twice daily for 4 days never exceeded 1 mm and should be of little clinical consequence.

In clinical trials in normal and glaucomatous humans, single 1.0-μg or repeated 0.5-μg PGF2α-IE doses lowered IOP by 20-25%, with only small or no effect on either outflow facility or aqueous pro-

Fig. 6. Refractive error after a single dose of PGF2α-IE. Data in top panels are mean ± SEM refractive error for n animals, each contributing one treated and one untreated eye; (□) PGF2α-IE-treated eyes, (○) untreated contralateral eyes. Data in bottom panels are mean ± SEM difference between treated and control eyes. Symbols on abscissa indicate significant difference between treated and control eyes by the two-tailed two-sample (top) or paired (bottom) t-test. *P < 0.05.
Fig. 7. Refractive error before PG treatment (day 0) and before and after the 7th dose of PGF$_{2\alpha}$-IE on the 4th day of twice-daily unilateral treatment. Time 0 occurs ~17 hr after the 6th PG dose (given at ~3:00 PM on day 3) and immediately before the 7th dose (given at ~8:30 AM on day 4). Data in left panels are mean ± SEM refractive error for n (# in 0.5-µg panel indicates n = 3) animals, each contributing one treated and one untreated eye; (●) PGF$_{2\alpha}$-treated eyes, (○) untreated contralateral eyes. Data in right panels are mean ± SEM difference between treated and control eyes. Symbols on abscissa indicate significant difference between treated and control eyes by the two-tailed two-sample (left) or paired (right) t-test. *P < 0.05, **P < 0.02, ***P < 0.01.
duction. This suggests that increased uveoscleral outflow is responsible for the IOP decrease in humans and subhuman primates. The PGF \(_2\alpha\)-IE dose–IOP response relationship for repeated drug administration has not yet been determined in the human; it is not known whether the extremely low IOP levels seen in monkeys will occur in normal or glaucomatous humans at repeated doses higher than those so far tested. The PGF \(_2\alpha\) may have especially great clinical potential in those glaucoma patients in which an IOP less than 10 mm Hg is deemed necessary as an alternative to full-thickness filtration surgery. However, much work remains before this drug will be ready for routine clinical use. The extraocular hyperemia and irritancy must be eliminated, and the intraocular safety margin related to blood–aqueous barrier breakdown and other signs of inflammation must be established for chronic dosing. Nonetheless, PGs are the most powerful topical ocular hypotensive agents yet developed and act by a mechanism different in kind and/or degree from all presently available drugs.

**Key words:** intraocular pressure, *Macaca fascicularis*, miosis, myopia, prostaglandin F \(_2\alpha\)

**Acknowledgments**

The authors thank Lynda J. Majors, Mary Ann Croft, and William C. Hubbard for expert technical assistance.

**References**