Noninvasive Observations on Eyes of Cats After Long-term Maintenance of Reduced Intraocular Pressure by Topical Application of Prostaglandin E₂

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Daily or twice daily prostaglandin E₂ (PGE₂) application to cat eyes was shown to maintain a reduced intraocular pressure (IOP) for several months without causing substantial flare or cellular response. We report now on detailed ophthalmic examinations performed on these cats after 5–9 months of such treatment (ie, after 150 to 250 unilateral PGE₂ applications; 100 µg/treatment per eye). A comparison of the treated and contralateral control eyes revealed no differences in the axial length of ocular compartments, in the biomicroscopic appearance of the lens, vitreous, retina, or optic nerve head, in the rate of light-induced pupillary constriction or in the wave form of the electroretinogram. The cell density of the corneal endothelium was not decreased, but the endothelial surface did contain a few small “dark spots.” A slight iridial heterochromia was generally apparent. In three of the cats PGE₂ application had a sialagogic effect that became a conditioned reflex. Cats tended to keep their lids closed after each treatment; lid closure was more prolonged in the PGE₂-treated eye than in the contralateral eye that received the same volume (50 µl) of vehicle solution. It is concluded that daily treatment with PGE₂, in doses sufficient to cause a maintained reduction in IOP, does have some side effects. However, none of these side effects are of sufficient importance to exclude the use of eicosanoids as potential anti-glaucoma agents.


It has been shown recently that PGs are effective ocular hypotensive agents in several mammalian species and that a reduced intraocular pressure (IOP) can be maintained for several weeks or months by daily or twice daily topical application of PGF₂α or PGE₂ to the eyes of rhesus monkeys or cats. In contrast only a temporary reduction of IOP can be obtained in rabbits due to the development of tachyphylaxis or subsensitivity to the ocular hypotensive effects of the topically applied PG. Furthermore, the rabbit eye is much more sensitive to PGs than feline or primate eyes and shows highly exaggerated ocular irritative and inflammatory responses and a very narrow margin between the dose of PG that reduces IOP and that causes an initial hypertension. For these reasons, cats were used for the study of the long-term ocular effects of PGs. Observations during the first part of that study were, however, limited to slit-lamp examinations of the anterior segment to avoid pharmacologic or mechanical manipulations of these eyes.

The present studies were undertaken to evaluate possible ocular side effects of PGE₂ after one eye of each of the six cats used in a 9-month IOP study had received 150 to 300 applications of a hypotensive dose of PGE₂. While daily PGE₂ application continued, specular microscopy of the corneal endothelium, ultrasonic measurements of ocular compartments, video-pupillography of the rate of light-induced pupillary constriction and/or biomicroscopic examination of the lens, vitreous, and fundus were performed. One of the cats was also used for an electroretinographic evaluation of retinal function. The results of these observations and some systemic reactions to the long-term PGE₂ treatment are reported.

Methods and results. Specular microscopy of the corneal endothelium: On day 152 and 196 of the previously described PGE₂ treatment, the cats were tranquilized with up to 20 mg/kg im Ketaset (ketamine hydrochloride; Bristol Labs). Specular microscopy was performed with a Koester wide-field specular microscope, using a 20x dipping-cone objective lens. Five to ten fields of each cornea were examined, and at least three photographs of each endothelium were taken. Visual examinations revealed no differences in cell density between the endothelia of the treated and control eyes, but dark spots were found, primarily at the endothelial cell junctions of the PGE₂-treated eyes, extending slightly into the anterior chamber. In two cats these dark spots were noted on the endothelium of both eyes. However, in the untreated eye only one or two such dark spots were found in five fields examined, while in the treated eye each field contained two to ten spots. Typical areas of the corneal endothelium of a treated eye and of the contralateral control eye are shown in Figure 1. The typical dark spot was about the size of a single cell but frequently covered parts of adjacent cells.

Cell densities of the corneal endothelium were measured on masked photographs of uniform mag-

0146-0404/83/0300/376/$1.05 © Association for Research in Vision and Ophthalmology

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Fig. 1. Photographs of specular microscopic fields of the typical corneal endothelium of a PGE$_2$-treated eye (panel A) showing some dark spots (arrows) and the contralateral control eye (panel B) of a cat on day 196 of the treatment period.

nification by having each of four people count five squares of a calibrated grid overlay (a $34 \times 34$ mm photographic grid area = $0.01$ mm$^2$ corneal endothelial surface) five times each. The mean of the cell counts ($2230 \pm 110$ for the treated eyes vs $2040 \pm 90$ for the control eye) showed a small but statistically significant difference ($P < 0.001$).

Evaluation of the appearance of the fundus and the crystallin lens: On day 257 of the PGE$_2$ treatment, 4 to 5 hr after PGE$_2$ application, one drop of 1% Cyclogyl (cyclopentolate hydrochloride, Alcon) was applied topically to both eyes. When complete mydriasis was observed, the animals were sedated with Ketaset. Repeated comparisons of the fundi of both eyes with an indirect ophthalmoscope revealed no perceptible differences. The vitreous of all eyes appeared clear and free of any pathology by ophthalmoscopy or slit-lamp examination and none of the lenses showed any opacities or cataractous changes.

Comparison of the rate of pupillary constriction in PGE$_2$-treated and contralateral eyes: On day 240, between 3 and 4 hr after the 228th PGE$_2$ treatment, each eye of each animal was monitored in a dark room, using infrared illumination, with a video camera (GBC total darkness camera). A bright light (650W 3400°K halogen source) equipped with a Schott KG4 filter to minimize its infrared content and a diffusor to prevent the animals from looking directly into the halogen source was placed at the distance from the animal's eye to obtain approximately 80% maximal pupillary constriction in normal cat eyes. The stimulus light was turned on for 4 sec, after which the eye was monitored for at least another 4 sec. The whole sequence was recorded at normal speed with a time-lapse Video Recorder (VEC, Model VS 7505). The tapes were played back on the same recorder, frame by frame or at a speed of $\frac{1}{34}$ of real time, and the nasotemporal pupillary diameter was measured with a ruler on the monitor screen.

Although the average resting pupillary diameter of
the PGE$_2$-treated eyes was 0.8 mm smaller than that of the contralateral control eyes, this difference is not statistically significant ($P > 0.05$). There was no difference between the treated and control eyes in the rate of light-induced pupillary constriction (Fig. 2). The mean changes in the pupillary diameter during the first second of stimulation were 4.5 ± 0.5 and 5.3 ± 0.7 mm in the experimental and control eyes, respectively, a difference which is not statistically significant ($P > 0.05$). Neither the experimental nor the control eyes redilated spontaneously immediately after the light was turned off, unless the animals were startled. Since this response could not be elicited reproducibly in all animals, no attempt was made to study pupillary redilation.

**Ultrasonic determination of ocular dimensions:** Between days 260 and 274 of PGE$_2$ treatment, the dimensions of the globe and its compartments were measured, using an ultrasonic biometric ruler (Model 300; Sonometrics, NY) equipped with a 5 MHz handheld contact transducer. For this procedure, the cats were anesthetized as described previously. The techniques, settings, and correction factors used were the same as those previously used for rhesus monkey eyes. The mean globe length (anterior surface of the cornea to retina) was $21.0 \pm 0.4$ mm and $20.3 \pm 0.3$ mm; the axial thickness of the lens was $7.5 \pm 0.1$ mm and $7.6 \pm 0.1$ mm and the depth of the anterior chamber was $4.8 \pm 0.2$ mm and $4.8 \pm 0.2$ mm for the PGE$_2$-treated and control eyes, respectively. None of the differences are statistically significant ($P > 0.5$).

**Normalcy of the electroretinogram:** Recording of the electrical responses of treated and control eyes to white ganzfeld flashes was performed by Dr. Peter Gouras of our department on one of the six cats on day 291 of the treatment, 3 hr after the application of 100 ng PGE$_2$ to the experimental eye of this animal. During light stimulation at a frequency of one flash per second, the responses obtained from the two eyes were essentially superimposable (Fig. 3). Similar results were obtained at three flashes per second.

**Other observations:** All cats closed their lids immediately after the application of PGE$_2$. On days 217 and 263, the duration of lid closure was monitored. The mean closure time for the control eyes was 2 to 3 min, while the PGE$_2$-treated eyes remained closed for up to 30 min. Thus, it appears that the solution containing PGE$_2$ caused more discomfort than did the vehicle solution alone. It should be noted, however, that this tendency to keep the PGE$_2$-treated eyes closed did not reflect the development of true blepharospasm since these cats would open their treated eyes a few minutes after PGE$_2$ application if they were startled.

Three of the six cats developed a salivagoric response during the latter part of the 9-month treatment period. Typically, this response was first observed in each animal a few seconds after the instillation of a PGE$_2$ dose. Within a few weeks after the first instance this response was noted, these cats began salivating routinely before the PGE$_2$ solution was actually applied to their eyes. In two cats, profuse salivation consistently began as soon as the pipet used to deliver the PGE$_2$ solution was brought close to their eyes. This continued for several minutes, even if the PGE$_2$ solution was not applied at that time.

A subtle heterochromia also became apparent in all six cats during the course of PGE$_2$ treatment. The iris of the treated eye appeared to be a slightly richer yellow than that of the control eye, with a slight tinge of orange.

**Discussion.** Because the corneal endothelium of
adult cats reportedly does not have the capacity to divide, the approximately 10% increase in cell density observed in the PGE2-treated eyes was a highly unexpected finding. The possibility that these cell counts were affected by the occurrence of dark spots (Fig. 1) cannot be ruled out. More studies will have to be done before the possibility that PG treatment increases endothelial cell density can be seriously considered, however, we can conclude that such long-term topical application of PGE2 does not lower endothelial cell density and causes only minimal morphologic changes in cats, a species that is considered to be a good model for the behavior of the human corneal endothelium.10

These dark spots most likely represent "inflammatory bodies"11 and may be the consequence of the low level flare and cellular response that were observed in the anterior segments of the PGE2-treated eyes.4 It should be pointed out, however, that the anterior chamber flare and cellular responses in these animals appeared to be partly due to frequent tonometry and/or the associated use of topical anesthetic.4 Thus, these dark spots may not represent a direct effect of PGE2 on the corneal endothelium.

The iridial heterochromia observed in these cats is unlikely to be of clinical concern because iridial function was not perceptibly affected, as indicated by the virtually identical rates of pupillary constriction in the treated and contralateral eyes. Furthermore, the change in iridial coloration was so slight that it was apparent only when the treated and untreated eyes were compared directly. The fact that light-induced pupillary constriction was not significantly affected in these cats and that their fundi did not reveal any abnormalities in coloration or vascular pattern, clearly indicates that PGE2 treatment did not have a deleterious effect on the retina. This conclusion is supported by the fact that the electroretinogram showed no differences between the control and PGE2-treated eyes.

In this first long-term study, PGE2 was used because earlier reports suggest that PGs of the E series have more severe adverse effects on the eye than PGs of the F series.5 However, except for a slight but consistent miosis, low-level flare, the occasional presence of cells in the anterior chamber,4 and the findings noted in this report, no other ocular or systemic side effects attributable to long-term PGE2 treatment were noted. All six animals gained some weight during the 9 months of treatment, as all cats do after they arrive at our animal care facility. Each of the three female cats in this long-term treatment group conceived, bore, and nursed healthy litters of kittens during the course of this treatment.4 It can be concluded that although long-term topical PGE2 treatment of the eye, in addition to its maintained ocular hypotensive effect, does produce some systemic and ocular side effects in cats, none of these side effects are severe enough to contraindicate the consideration of this or other eicosanoids for the treatment of ocular hypertension and glaucoma. However, because there are considerable species variations in the responses of the eye to chemical irritants or inflammatory agents, extensive testing on primate eyes, and ultimately on human eyes, will be required before the efficacy and safety of topical application of eicosanoids or their derivatives for the treatment of glaucoma patients can be evaluated.

Key words: cat, prostaglandin, prostaglandin E2, ocular hypotension, intraocular pressure, glaucoma, pupillary constriction, iridial heterochromia, corneal endothelium, electroretinogram, lens

Acknowledgments. The authors wish to thank Dr. Peter Gouras for performing the electroretinography, Dr. Charles Koester for the loan and maintenance of the specular microscope, Dr. John Pike of The Upjohn Company for the generous supply of PGE2, and Santos Rodriguez for his technical assistance.


References
7. Klein EM and Bito LZ: Species variations in the pathophys-
were increased in patients with a history of previous surgery and eyes with anterior uveitis. We found that IgG and complement increased proportionately in inflamed aqueous humor. Finally we compared the ratios of IgG to complement in normal and inflamed aqueous humor as done by Chandler and associates to determine if levels of IgG and complement varied proportionately or disproportionately.

Materials and methods. Samples of venous blood and aqueous humor were obtained from 14 patients. Group 1 consisted of venous blood and aqueous humor samples from eight patients with cataracts and anterior chamber inflammation (Tables 2A, B). Prior to collection of aqueous humor, patients received lid and retrobulbar injections of 2% mepivacaine hydrochloride mixed with an equal volume of 0.75% bupivacaine hydrochloride. Approximately 0.15 ml of aqueous humor was collected from all patients in a 1-cc tuberculin syringe with a 30-gauge needle. The anterior chamber was entered through the trephine groove in patients undergoing cataract extraction and through the temporal limbus in patients undergoing corneal transplantation, through a superior limbal groove in patients 12 and 14 undergoing corneal transplantation, through a superior limbal groove in patients 1–8 and 10 undergoing cataract extraction and through the temporal limbus in patients 9, 11, and 13.

The aqueous samples were placed in ice. Venous blood samples were also collected from all patients at the time of surgery. Both the aqueous humor and venous blood samples were picked up immediately by a technician. The venous blood was allowed to clot and then centrifuged at 1200 rpm for 10 min to separate the serum from the clot. The aqueous humor and serum were stored at −70°C. Serum and aqueous humor from the same patient were analyzed on the same day with the same reagents.