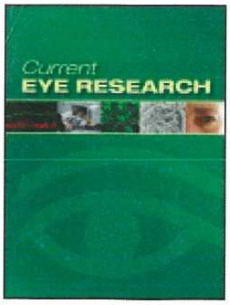

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Pharmacological testing in the laser-induced monkey glaucoma model

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ABSTRACT

Glaucoma was induced in cynomolgus monkeys by photocoagulating the trabecular meshwork with the argon laser. Repeat treatments were often necessary and wide intraocular pressure fluctuations were characteristic.

Baseline intraocular pressure was measured with a calibrated pneumatonometer hourly for six hours. On a succeeding day a baseline measurement was made, 50 µl of the drug to be tested applied, and six hourly measurements of intraocular pressure repeated. The effects on intraocular pressure of timolol, epinephrine, pilocarpine, vanadate, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), forskolin, and corynanthine were tested in at least eight eyes. Significant ($p < 0.05$) reductions of intraocular pressure were produced by 0.5% timolol, 2% epinephrine, 4% pilocarpine, 1% vanadate, 500 µg of $PGF_{2\alpha}$ and 1% forskolin. Five per cent corynanthine produced no significant lowering of intraocular pressure.

Tonography revealed an increased outflow facility associated with the reduction of intraocular pressure 2 hours after the administration of 4% pilocarpine.

This glaucoma animal model may be useful in investigating agents that lower intraocular pressure by a variety of mechanisms.

INTRODUCTION

The search for animals with different types of spontaneous glaucoma has revealed a few models in rabbits, dogs, chickens, and primates (1). The general disadvantage of most animal models, with the exception of primates, is that the iridocorneal angle anatomy is different from that of the human. Although all species seem to have a continuous endothelial lining of the aqueous outflow channels, there are major differences in the presence of pectinate ligaments, a limited ciliary body musculature, and a scleral venous plexus instead of Schlemm's canal (2).

Historically, investigators have tried to create animal models of glaucoma since as early as 1870. Some methods have failed and others produced transient or prolonged elevations of intraocular pressure. Glaucoma has been produced in rabbits by the injection of 1% kaolin into the anterior chamber, and by encircling the globe with cotton threads or rubber bands (1,3). Kupfer (4), by inserting a polyethylene tubing into the angle of the anterior chamber of the rabbit eye, produced an elevated intraocular pressure within 24 hours which remained elevated for at least 3 months. Samis (5) and Kazdan (6) created glaucoma in rabbits by blocking the angle of the anterior chamber with methyl cellulose. Hamasaki and Ellerman (7) found that injection of alpha-chymotrypsin directly into owl monkey eyes caused an elevation in intraocular pressure. This technique has also been used successfully in rhesus monkeys (8) and rabbits (9). Intraocular pressure elevations lasting from 2 to 42 days have been produced in squirrel and cynomolgus monkey eyes by anterior chamber injections of autologous, fixed red blood cells (10).

Gaasterland and Kupfer (11) described a new method for the production of sustained, elevated intraocular pressure in the rhesus monkey by repeated, circumferential argon laser photocoagulation of the mid-trabecular meshwork. This technique caused a sustained elevation of intraocular pressure, reduction of outflow facility, and retinal and optic nerve changes similar to those seen in human chronic, open-angle glaucoma. This model has been available for studies for 11 years. Quigley and Hohman (12) treated the trabecular meshwork of cynomolgus monkey eyes with the argon laser by a variety of protocols in an attempt to cause moderate, consistent intraocular pressure elevation. This was achieved most satisfactorily with deliveries of

0.5 to 1.0 seconds and a total energy of at least 50 joules. By light and electron microscopy, the trabecular beams were blunted, and scattered synechiae were present. The disc changes in these experimental eyes have been similar to those previously described in human eyes with glaucoma (13,14).

A satisfactory animal model has not been available for the investigation of the effect of a number of commonly used clinical and experimental drugs for the treatment of glaucoma. The experimental model of glaucoma in rabbit induced by the posterior chamber injection of alpha-chymotrypsin has been studied as to the effects of timolol, epinephrine, norepinephrine, isoproterenol and propranolol on intraocular pressure (15). The effect of topical pilocarpine on intraocular pressure has been studied in normotensive and glaucomatous beagles (16). We carried out a study of the effect of topically applied timolol, epinephrine, pilocarpine, vanadate, prostaglandin $F_{2\alpha}$, forskolin and corynanthine on intraocular pressure in the laser-induced monkey glaucoma model.

MATERIALS AND METHODS

Eight cynomolgus monkeys weighing 3 to 4 kg, were used. Baseline examination showed eyes with normal anterior chamber angles, normal intraocular pressure, normal outflow facility, clear ocular media, and normal optic nerveheads. Following baseline examination, 13 eyes (bilateral in 5 monkeys and unilateral in 3 monkeys) were treated using the argon laser (Coherent Radiation Model 9900, U.S.A.). Ketamine hydrochloride was injected intramuscularly (5-10 mg/kg) for sedation during laser therapy. The eyes were treated with topical proparacaine 0.5% and photocoagulated using a single mirror goniolens specially made to cynomolgus monkey specifications (Ocular Instruments, Bellevue, Washington, U.S.A.). Between 50 and 130 50-micron spots of 1000-1500 mW power and 0.5 seconds exposure time were applied to the mid-portion of the trabecular meshwork for 360°. Fundus examinations and intraocular pressure measurements were repeated every seven days. Retreatment of the trabecular meshwork with laser was done if the intraocular pressure remained normal.

For the intraocular pressure measurements, the monkeys were kept in a sitting position in specially designed chairs throughout each experiment. The intraocular pressure was measured with a Model 30R pneumatonometer (Digilab, Inc., Cambridge, Massachusetts, U.S.A.) in animals lightly anesthetized with ketamine hydrochloride, 3-5 mg/kg given intramuscularly, about 5 minutes before each measurement. The instrument was calibrated by the manufacturer for humans and the verifier was used to check the tonometer calibration. Topical 0.5% proparacaine anesthesia was instilled prior to all intraocular pressure measurements.

The drugs employed included sodium chloride 0.9%, timolol maleate 0.5% (Merck Sharp & Dohme, West Point, Pa., U.S.A.), L-epinephrine HCl 2% (Alcon Laboratories, Inc., Fort Worth, Texas, U.S.A.), pilocarpine HCl 4% (Alcon, Humacao, Puerto Rico, U.S.A.), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 5 mg/ml (The Upjohn Co., Kalamazoo, Michigan, U.S.A.), vanadate ($NaVO_3$) (E. Merck, Darmstadt, Germany) prepared as a 1% solution in distilled water, 10% DMSO, and 5% Tween 80, forskolin (Calbiochem Behring Co., La Jolla, Calif., U.S.A.) prepared as a 1% suspension in isotonic buffered saline containing 0.5% methylcellulose, and corynanthine (Sigma Co., St. Louis, Mo., U.S.A.) prepared as a 5% solution in distilled water. The vanadate, forskolin, and corynanthine were prepared fresh daily prior to topical ocular delivery. We did not use vehicles in the control measurements for vanadate and forskolin because the vehicles had no effects on intraocular pressure in normal cynomolgus monkey eyes in our previous studies. For all experiments a 50 µl drop size was used. One drop (50 µl) was used of sodium chloride, timolol, L-epinephrine, pilocarpine, vanadate, forskolin, and corynanthine, and two drops were used of

$PGF_{2\alpha}$ three to five minutes apart.

The glaucomatous monkeys underwent baseline (control day) and drug treated (treated day) 6-hour diurnal curves, the intraocular pressure being recorded at 9:30 a.m., 10 a.m., 10:30 a.m., 11:30 a.m., 12:30 p.m., 1:30 p.m., 2:30 p.m., and 3:30 p.m. The baseline diurnal curve served as a control and was made 1 or 2 days before the experimental (treated) diurnal curve. On the experimental day baseline intraocular pressure was measured at 9:30 a.m. Each drug was administered to both eyes of the monkey immediately after the 9:30 a.m. measurement (0 hour). Included in this study were those monkey eyes in which the intraocular pressure was 22 mm Hg or more in both the baseline diurnal curve and the drug treated baseline intraocular pressure measurements. A two-week washout period was employed between testing each drug on the same monkey. Occasionally the same eye was used twice to test the same drug.

Tonography was performed after using 4% pilocarpine with an EDT-103 tonography unit (Alcon). Baseline outflow facility was determined between 9 a.m. and 10 a.m., 2 hours before administration of 4% pilocarpine. Tonography was repeated 2 hours after drug administration. Outflow facility values were approximated from the 1955 Friedenwald tables.

We employed two methods to analyze the changes of intraocular pressure after drug administration, because of the wide intraocular pressure fluctuations in the glaucomatous monkey model. Method 1: The intraocular pressures on the treated day were compared to that on the control day. Method 2: The differences in intraocular pressures at intervals after therapy between the treated and control day measurements were compared to the initial (0 hour) differences between the treated and control day values.

RESULTS

Elevated intraocular pressure (IOP) was achieved in all 13 eyes treated with the argon laser. Treatment was to the mid-trabecular meshwork and caused immediate blanching, bubble formation, and pigment scatter. Occasionally, a small hyphema was noted. The intraocular pressure often fell the week following treatment and rose on the subsequent week or fell if the treatment had been inadequate. Three eyes maintained a raised intraocular pressure after only one treatment session of 56-72 joules but most eyes needed 2-5 treatment sessions. Wide pressure fluctuations were noted in all monkey eyes as previously reported by Pederson and Gaasterland (13). Optic disc cupping was ultimately noted in 8 out of the 13 eyes during the course of this study. All eyes showed fixed mydriasis which may be due to laser-induced damage of ciliary nerves that pass through the ciliary body and innervate the iris sphincter (13).

The effects of sodium chloride, timolol, epinephrine, pilocarpine, vanadate, $PGF_{2}\alpha$, forskolin, and corynanthine on the intraocular pressure in this glaucomatous monkey model are shown in Table 1.

Intraocular pressures before a single instillation of these drugs (0 hour) were similar in treated and control day measurements in all groups. Sodium chloride 0.9% instillations had no significant effect on intraocular pressure, comparing control and drug treated 6-hour diurnal curves.

The 0.5% timolol significantly ($p < 0.05$) lowered intraocular pressure from 3 to 6 hours after instillation. The maximum effect occurred 5 hours after drug administration, and persisted at this level for an additional 1 hour, at which time the experiments were terminated.

Topical application of 2% epinephrine to the monkey eyes produced a significant ($p < 0.05$) decrease in intraocular pressure occurring between 0.5 and 6 hours after drug administration. The maximum reduction in intraocular pressure, occurred 1 hour after drug application.

Four percent pilocarpine produced a significant ($p < 0.05$) reduction of intraocular

Table 1: Effect of Sodium Chloride, Timolol, Epinephrine, Pilocarpine, Vanadate, $PGF_{2\alpha}$, Forskolin, and Corynanthine, Topically Administered, on the Intraocular Pressure in Glaucomatous Cynomolgus Monkey Eyes

Groups†	Eye No.	Mean intraocular pressure (mm Hg ± S.E.) 0 Hr¶	0.5 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
Control	12	36.0±3.4	35.8±3.6	35.4±3.4	34.7±3.2	35.5±3.2	35.0±3.0	32.9±2.8	33.7±3.3
Sodium chloride 0.9%	12	34.0±3.6	33.7±3.8	33.4±3.8	33.5±3.8	33.0±3.8	32.9±4.0	33.1±4.2	32.4±4.3
Control	8	35.9±4.4	34.1±4.4	32.5±4.4	28.9±3.8	29.6±3.9	29.3±3.8	27.8±3.9	28.0±4.0
Timolol, 0.5%	8	36.8±4.6	34.6±4.3	29.9±4.4	25.4±3.7	22.9±4.1§	21.4±4.5§	19.8±3.7§	20.9±3.7§
Control	8	38.3±3.3	39.5±3.5	40.0±3.3	39.5±3.8	39.8±3.9	39.0±4.0	38.0±4.3	35.6±4.5
Epinephrine, 2%	8	34.6±3.5	28.4±3.6§	28.8±4.2§	29.5±4.6§	30.3±4.6§	29.8±4.8§	28.0±4.9§	26.3±4.6*
Control	14	35.0±3.4	34.4±3.3	34.1±3.2	34.1±2.9	33.6±3.0	33.6±2.9	32.7±2.9	31.9±3.1
Pilocarpine, 4%	14	33.7±3.2	25.4±3.2§	23.1±3.3§	23.5±3.2§	24.4±3.3§	23.9±3.3§	24.2±3.2§	24.0±3.4*
Control	8	39.1±3.8	40.1±3.9	41.0±3.7	42.1±3.4	42.9±3.4	42.5±3.6	41.1±3.7	39.4±3.7
Vanadate, 1%	8	37.3±4.1	36.3±3.8*	37.6±3.9*	35.6±4.4	34.3±4.7*	34.9±4.8	34.6±4.4	35.6±4.3
Control	9	32.2±3.9	29.7±3.4	28.6±3.3	32.1±4.0	30.4±3.8	28.7±4.1	27.3±3.4	26.4±3.0
$PGF_{2\alpha}$, 0.005%	9	32.7±2.8	32.1±3.0	28.4±3.5	27.1±3.7§	25.9±3.9§	23.8±3.7§	24.7±3.6	24.7±3.4
Control	9	33.0±3.8	32.3±3.9	31.9±3.6	33.9±3.7	34.1±3.9	32.4±3.8	29.8±3.6	28.7±3.2
Forskolin, 1%	9	33.6±3.2	29.1±4.0	27.8±4.1§	30.1±4.4	30.0±4.3	29.4±4.4	29.2±4.3	28.7±4.5
Control	14	34.6±3.0	33.8±2.9	33.0±2.7	31.6±2.9	31.9±3.0	32.0±3.4	32.9±3.5	33.0±3.6
Corynanthine,5%	14	32.1±3.1	31.1±3.2	29.3±3.5	29.3±3.6	29.1±3.7	30.5±3.8	30.4±5.5	31.6±4.1

†Both eyes of the drug-treated group were treated with 50 µl (sodium chloride, timolol, epinephrine, pilocarpine, vanadate, forskolin, and corynanthine) or 100 µl ($PGF_{2\alpha}$) of the indicated concentration.

¶Time after administration

*Significantly different ($p < 0.05$) from the control day measurements at corresponding intervals, paired t-test.

§Significantly different ($p < 0.05$) from the control day measurements at corresponding intervals and the differences between the treated and control day measurements significantly different ($p < 0.05$) from the initial (0 hr) differences between the treated and control day measurements, paired t-test.

pressure at 0.5 to 6 hours after the drug administration. The maximum effect occurred at 1 to 2 hours.

One percent vanadate significantly ($p < 0.05$) decreased intraocular pressure in the treated eyes at 0.5, 1, and 3 hours only comparing control and treated day measurements. The maximum reduction occurred 3 hours after drug administration.

Topical application of 500 µg of $PGF_{2\alpha}$ to the monkey eyes produced a significant ($p < 0.05$) decrease in intraocular pressure occurring between 2 to 4 hours after drug administration. The levels of intraocular pressure reduction were similar at 2, 3, and 4 hours.

One percent forskolin significantly ($p < 0.05$) lowered intraocular pressure only at 1 hour after drug administration.

There was no significant ($p > 0.1$) difference in intraocular pressure between treated and control eyes after 5% corynanthine administration.

In 13 eyes of 8 glaucomatous monkeys, 2 hours after a topical administration of 4% pilocarpine, the mean intraocular pressure was significantly ($p < 0.001$) reduced in the treated eyes as compared to the baseline value, and the mean outflow facility was significantly ($p < 0.01$) increased as compared to the baseline value (Table 2). Before laser photocoagulation, the intraocular

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