Intraocular Pressure in Rabbits by Telemetry II: Effects of Animal Handling and Drugs

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PURPOSE. To measure under carefully controlled conditions the effects of pharmacologic agents for glaucoma.

METHODS. Rabbits were outfitted in one eye with an implantable telemetric pressure transducer and monitored for several months under controlled conditions of light/dark and handling. Effects of tonometry, handling, water drinking, and instillation of topical ophthalmic medications on intraocular pressure were recorded during each 24-hour day/night cycle.

RESULTS. Pneumatonometry, animal handling, and water drinking all had an effect on intraocular pressure that in many instances was of the same magnitude as the effects of pharmacologic agents. Dorzolamide and timolol caused a sustained reduction of intraocular pressure during the nocturnal period. Epinephrine had a biphasic effect, causing an immediate pressure elevation followed by a prolonged depression. Apraclonidine, latanoprost, and pilocarpine had no measurable effect.

CONCLUSIONS. Continuous telemetric measurement of intraocular pressure in rabbits permits the measurement of uncontrollable artifacts that occur with tonometric measurements and animal handling. If environmental conditions are rigidly controlled, this method is very sensitive for detecting therapeutic effects of candidates for ocular hypotensive drugs. When healthy animals are used, the method appears to be more sensitive for drugs that affect aqueous humor formation than for drugs that affect aqueous humor outflow resistance. (Invest Ophtalmol Vis Sci. 1998;39:2485–2489)

A considerable body of information about mammalian aqueous humor physiology has been acquired from experimental studies of rabbits. Until recently, most of the measurements of intraocular pressure in this animal were carried out by manometry or by tonometry. With these methods, it has always been necessary to disturb the animal in some way at the moment instantaneous intraocular pressure is measured.

McLaren and coworkers1,2 and Schnell and coworkers3,4 used a telemetric transducer system to permit continuous measurement of intraocular pressure over long periods of time in conscious and undisturbed animals. This method permits study under controlled experimental conditions without interference by the tonometric procedure itself. Using this system, these investigators confirmed the circadian rhythm of intraocular pressure in this species and demonstrated that the telemetric system is capable of measuring intraocular pressure effects of a variety of disturbances such as application of tonometry, intravenous infusion of osmotic agents, and topical application of timolol. The method is generally applicable to measurements of phenomena that occur in seconds or trends that extend over many months.

The present study explores additional advantages of the telemetric system as a screening tool for the intraocular pressure effects of pharmacologic agents. The study shows that artifacts caused by handling, tonometry, circadian cycle, and water drinking can be eliminated and that pharmacologic effects can be detected, even with the use of very small numbers of animals.

METHODS

Five pigmented rabbits weighing between 2 kg and 2.5 kg were studied in a protocol approved by the Institutional Animal Care and Use Committee of the Mayo Clinic and in conformance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Each animal was outfitted with an implantable pressure monitor (model TA111PA-C40; Data Sciences International, St. Paul, MN) according to the surgical procedure described previously.2 Briefly, the body of the transducer was implanted in a musculocutaneous pocket in the nape of the animal’s neck, the connecting tubing was tunneled beneath the skin into the orbit, and the tip of the tubing was inserted into the anterior chamber through a beveled corneal incision. After a period of healing and stabilization, each animal was ready for pressure studies using the caging, telemetric, and computer system described previously.2
Effects of environmental disturbances on intraocular pressure in rabbits. —, day of disturbance at time indicated by arrow; ...., undisturbed day. (A) Effect of pneumatonometry on intraocular pressure in five animals. Pressure rose and remained elevated for nearly 1 hour after tonometry. Intraocular pressures were averaged at 10-minute intervals; in all other graphs, pressures were averaged at 30-minute intervals. (B) Transferring an animal from one cage to another elevated intraocular pressure for 1 hour after the animal was disturbed on each of 2 days (one animal). (C) Effects of drinking water after a 24-hour period of water deprivation. Just after animals began to drink, intraocular pressure rose and remained elevated for several hours (mean of five animals).

All animals were kept in an inner room in which the lighting could be controlled and where entry of animal care personnel was controlled and kept to a minimum. Lights were turned on for 12 hours between the hours of midnight and noon, and then turned off for 12 hours between noon and midnight. All times reported here are both time of day and circadian time (at circadian time 00:00, lights are turned on).

Intraocular pressure was measured at 50 samples per second for 15 seconds, and the mean pressure during this interval was saved. This measurement was repeated every 2.5 minutes, and every group of 12 measurements was averaged to limit our observations to slow changes that are of greatest interest when screening medications for pressure-lowering effects. These 30-minute averages were stored in the computer database (for purposes of demonstration, groups of 4 measurements, taken over 10 minutes, were averaged in some examples presented here). Intraocular pressures measured between 2:00 AM and 6:00 AM, the nadir of the circadian cycle, were further averaged. This average was subtracted from all intraocular pressures to normalize each cycle relative to this nadir and to eliminate any drift in the offset of the transducer. This procedure eliminated the need for frequent manometric or tonometric calibrations. (Previous studies have shown that the gain of these transducers is very stable even though their baseline can drift slowly over several months.) Baselines of the circadian cycle for the drug studies were calculated by averaging the normalized 30-minute measurements on 3 days when a placebo had been administered. The placebo eyedrop was instilled at the same time of day and in the same way as each of the subsequent experimental drugs. In this article, mean intraocular pressures for a group of animals are presented as the normalized pressure, and data from an individual animal are not normalized.

Procedures

Pneumatonometry. Animals were removed from their cages during the light period, and intraocular pressure was measured with a pneumatonometer (model 30 Classic; Mentor O&O, Norwell, MA) three to five times within 3 minutes after instillation of proparacaine 0.5% (Ophthetic; Allergan, Homigueros, Puerto Rico).

Animal Handling. Each animal was removed from its cage, the receivers and associated wiring were transferred to a clean cage, and the animal was placed in the clean cage. This procedure took approximately 5 minutes per animal.

Water Drinking. The source of drinking water to each cage was removed for 24 hours. Water was then restored, and the animals were allowed to drink ad libitum.

Topical Ophthalmic Drugs. Intraocular pressure was measured after administering several classes of topical medications known to reduce intraocular pressure in humans: dorzolamide hydrochloride 2% (Trusopt; Merck & Co., West Point, PA), epinephrine hydrochloride 2% (Epifrin; Allergan Pharmaceuticals, Irvine, CA), timolol maleate 0.5% (Timoptic; Merck & Co.), apraclonidine hydrochloride 0.5% (Iopidine; Alcon Laboratories, Ft. Worth, TX), latanoprost 0.005% (Xalatan; Pharmacia, Kalalamoo, MI), and pilocarpine hydrochloride 2% (Pilocar; IOLAB Corporation, Claremont, CA). Artificial tears (HypoTears; IOLAB Corporation) were administered as a placebo. Unless otherwise indicated, all drugs were instilled in the lower cul-de-sac of the eye in three doses, 50 µl each, separated by 2 minutes. All drugs were administered between 11:30 AM and 12:00 noon.
TABLE 1. Effects of Topical Medication on Intraocular Pressure in Four Pigmented Rabbits

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Drug</th>
<th>1:30 to 2:30 PM</th>
<th>IOP Difference</th>
<th>3:30 to 4:30 PM</th>
<th>IOP Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>—</td>
<td>8.5 ± 1.3</td>
<td>—</td>
<td>7.0 ± 1.0</td>
<td>—</td>
</tr>
<tr>
<td>Dorzolamide, 0.2%</td>
<td>Topical CAI</td>
<td>3.5 ± 1.8</td>
<td>−5.0 ± 1.5*</td>
<td>3.5 ± 1.2</td>
<td>−3.6 ± 0.8*</td>
</tr>
<tr>
<td>Epinephrine, 2%</td>
<td>β-Adrenergic agonist</td>
<td>3.7 ± 1.9</td>
<td>−4.8 ± 1.6*</td>
<td>3.6 ± 1.9</td>
<td>−3.4 ± 2.2t</td>
</tr>
<tr>
<td>Timolol, 0.5% for 5 days</td>
<td>β-Adrenergic antagonist</td>
<td>4.9 ± 0.8</td>
<td>−3.5 ± 1.2*</td>
<td>5.0 ± 1.4</td>
<td>−2.0 ± 2.0 (−4.1)</td>
</tr>
<tr>
<td>Apraclonidine, 0.5%</td>
<td>α-Adrenergic agonist</td>
<td>5.2 ± 0.8</td>
<td>−3.3 ± 1.0*</td>
<td>5.6 ± 0.4</td>
<td>−1.4 ± 0.9t</td>
</tr>
<tr>
<td>Latanoprost, 0.005%</td>
<td>Prostaglandin analogue</td>
<td>7.0 ± 0.9</td>
<td>−1.5 ± 1.0†</td>
<td>6.6 ± 0.8</td>
<td>−0.4 ± 0.4 (−0.8)</td>
</tr>
<tr>
<td>Pilocarpine, 2%</td>
<td>Cholinomimetic</td>
<td>7.4 ± 1.5</td>
<td>−1.0 ± 2.1 (−4.3)</td>
<td>6.0 ± 1.2</td>
<td>−1.1 ± 1.5 (−3.1)</td>
</tr>
</tbody>
</table>

All intraocular pressures (IOP) are given as normalized mean ± SD in mm Hg. Numbers in parentheses are minimum differences that could be detected with 90% power. IOP difference indicates mean difference with IOP on placebo day at same time. Probability values are given for decreases that were significant, one-sided paired t-test.

*P < 0.05.
†P < 0.05.

30 minutes or less before the onset of the dark periods. Four of the five animals had stable implants throughout the period of drug testing, and their data are presented.

Statistical Analysis

If any of the topical medications were to have a detectable effect, it would most likely have occurred when the intraocular pressure was normally high and at a time when drug concentration in the ocular tissues was near its maximum. We limited our statistical analysis to the average pressure during two intervals: 1:30 PM to 2:30 PM and 3:30 PM to 4:30 PM. These intervals represent times after drug instillation (2-3 hours and 4-5 hours) at which drug concentrations in the aqueous humor should have been maximal and when any pressure-lowering effects should have been measurable. They were also immediately after the natural nocturnal pressure rise when intraocular pressure was at a maximum. The averaged pressures were compared with the averaged pressures during the same intervals on the days when the placebo had been administered. Significance of a pressure decrease was determined by using a one-sided t-test for paired samples; P ≤ 0.05 was considered statistically significant.

RESULTS

Pneumatonometry

After pneumatonometry, intraocular pressure was disrupted from the normal pattern for a period that lasted from a few minutes to several hours, depending on the animal. The mean intraocular pressures of five animals are shown in Figure 1A. Immediately after pneumatonometry, intraocular pressure increased by approximately 3 mm Hg and remained elevated for approximately 1 hour. In some animals intraocular pressure then dropped to 2 mm Hg below normal pressure for approximately 1 hour. Thereafter, intraocular pressure returned to and followed the normal circadian rhythm.

Animal Handling

When animals were removed from their cages and returned to clean cages, the response was similar to the transient pressure changes after pneumatonometry; intraocular pressure changed from normal for a period lasting from a few minutes to several hours depending on the individual animal. A strong long-lasting response was shown in one animal on 2 days and is shown in Figure 1B. Both days were consistent; intraocular pressure rose to a maximum within 1 hour after animals were transferred to clean cages and then dropped to below normal for approximately 2 hours. Intraocular pressure returned to the normal pattern after 5 hours.

Water Drinking

After the water supply was restored, animals immediately began to drink, and intraocular pressure increased rapidly, by approximately 7 mm Hg (Fig. 1C). The intraocular pressure remained elevated for approximately 4 hours. Thereafter, it followed the rhythm of the previous 5 days.

Effects of Topical Ophthalmic Drugs

The mean normalized intraocular pressure (difference between intraocular pressure and mean pressure between 2:00 AM and 6:00 AM) during the two intervals after drug administration are given in Table 1. The mean difference between pressure on the drug-treatment day and pressure on the placebo-treatment day is also given along with the significance of the decrease. For differences that were not statistically significant, Table 1 shows the precision of the t-test, the smallest difference one could expect to find, with a 90% power.

Dorzolamide. The instillation of dorzolamide was followed by a lowering of intraocular pressure relative to the placebo period. The effect was statistically significant during both intervals (Fig. 2A).

Epinephrine. The instillation of two 50 μl drops of epinephrine was followed initially by a transient increase in pressure that lasted approximately 1 hour. Thereafter, the intraocular pressure decreased for several hours, relative to pressure on placebo-treatment days (Fig. 2B).

Timolol. Timolol when given in one dose decreased intraocular pressure during the dark phase (Fig. 2C). The change in intraocular pressure on a placebo-treatment day was significant during the early test interval. For the later interval, the mean pressure difference was −2.0 ± 2.0 mm Hg and was not statistically significant. When three drops of timolol were given
FIGURE 2. Effects of topical ophthalmic preparations on intraocular pressure in four rabbits (- - - - , placebo day; ---, test day, arrow indicates time of drug or placebo instillation). (A) Dorzolamide 2% blunted and delayed the nocturnal rise of intraocular pressure. (B) Epinephrine 2% caused an immediate transient rise of intraocular pressure followed by a prolonged reduction. (C) Timolol 0.5% reduced the nocturnal rise of intraocular pressure. No difference was seen between the effect of a one-time dose and a dose repeated daily for 5 days. (D) Apraclonidine 0.5% caused no measurable change. (E) Latanoprost 0.005% caused a decrease of 1.5 mm Hg during the early interval but no statistically significant decrease during the later interval. (F) Pilocarpine 2% caused no statistically significant effect on the intraocular pressure.

daily for 5 days, intraocular pressure decreased by approximately the same amount as it did after 1 day’s dose, and the change was significant during both test intervals.

**Apraclonidine.** The instillation of apraclonidine 0.5% was not followed by any significant change in the intraocular pressure compared with the normal circadian rhythm when placebo was given (Fig. 2D).

**Latanoprost.** Administration of latanoprost caused a lowering of intraocular pressure by approximately 1.5 mm Hg during the early interval. The decrease during the second interval was not statistically significant (Fig. 2E).

**Pilocarpine.** Pilocarpine 2% caused a pressure decrease of approximately 1 mm Hg, but in four animals the effect was not statistically significant (Fig. 2F).
DISCUSSION

This study shows that the rabbit eye is very sensitive to a number of disturbances and pharmacologic agents that are commonly used to treat glaucoma. This method of continuous monitoring, if used properly, is a powerful tool to discover and explore the effects of some potentially useful compounds, even with a small sample of animals. In this study, the average SD of differences in intraocular pressure at the same time of day on different days was 1.5 mm Hg. With only four animals one should have a 90% chance of finding a pressure difference of 3.4 mm Hg (a 19% decrease from a baseline of 18 mm Hg) between drug treatment and placebo treatment days. If the number of animals was increased to six, one would have the same chance of finding a difference of 2.4 mm Hg (a 13% decrease from baseline of 18 mm Hg). With four animals we were able to detect decreases in intraocular pressure after treatment with timolol, dorzolamide, epinephrine, and latanoprost, confirming the results of Percicot and coworkers. 4

Useful results with this technique can only be obtained when the environmental conditions of the animals are carefully controlled. This experiment shows that several environmental standards must be maintained and that several principles must be followed to obtain useful information from the experiment. First, the circadian cycle of intraocular pressure is closely linked to the light schedule, and a change in the time of lighting can affect the intraocular pressure. One must be certain that the lighting schedule is not inadvertently changed during the course of a study. A shift of 6 hours might require as much as 2 weeks before an animal's circadian rhythm has realigned with the new light schedule. 2

Second, the timing of drug instillation relative to the light cycle is critical, and comparisons between test- and placebo-treatment days must be made at the same time in the circadian cycle. 4 In this experiment we chose to administer the agents just before the nocturnal rise in pressure. Rabbits are more active during the dark phase than they are during the light phase. Instilling medications just before the onset of dark is analogous to administering medications in the morning in humans; this is a time when an oculohypotensive drug would most likely affect pressure by blunting the normally sharp rise or reducing the nocturnal plateau. The pressure-lowering effects might have been different if the drugs had been instilled later in the nocturnal period or during the diurnal low-pressure period. One could study the potential origin of this circadian cycle by examining the effects of drugs with known pharmacologic actions after they are administered at specific times in the cycle. 2

Third, in addition to lighting, other environmental conditions must be constant. We demonstrated that the water supply to the animals must be continuous. Any disruption in fluid balance can alter intraocular pressure and mask or enhance a pharmacologic effect. An otherwise innocuous change from the daily routine can disrupt the normal intraocular pressure cycle for a period ranging from minutes to hours. 

Finally, one must be aware that handling an animal (for example, during tonometry or transfer to a clean cage) can change intraocular pressure and that this change can last for several hours beyond the interaction. The response to handling is much stronger in some animals than it is in others. It is typically an increase followed by a decrease from the normal pressure. The variable response in a group of animals would increase the variability of measurements and reduce the power for a given number of animals.

Many of these artifacts can be avoided by using a telemetric system to measure intraocular pressure. Once the test agent has been instilled, the animal need not be disturbed to measure and collect intraocular pressure. The ambient light can be monitored through a photocell and a separate channel on the recording system to verify that the lighting cycle remains constant.

Of the drugs we tested, those known to be suppressors of aqueous humor formation in humans, except for apraclonidine, had easily detectable pressure-lowering effects in a very small sample of normal rabbits. Our results confirmed the findings of Percicot and coworkers, who measured the therapeutic effects of timolol and dorzolamide and who noted the transient elevation of intraocular pressure after topical epinephrine. 4 However, we found minimal decreases of intraocular pressure after administration of drugs that are known to affect aqueous humor outflow. In our small sample, the sensitivity of the method may have been enhanced by rendering the animals hypertensive, as has been done in other studies that used tonometry for measuring intraocular pressure2-10 and, more recently, in studies that used implanted tonometers. 3,4 One must use care in interpreting intraocular pressure changes in rabbits and in extrapolating these results to humans. The rabbit seems to be a better model for drugs that suppress inflow than it is for drugs that enhance outflow facility. Regardless of the system used for intraocular pressure measurement, species differences in drug effects will remain a challenge to drug discovery programs for human disease.

References