Method for the Noninvasive Measurement of Intraocular Pressure in Mice

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PURPOSE. To evaluate the applicability of rebound tonometry for measurement of IOP in the mouse eye.

METHODS. An induction–impact (I/I) tonometer, which operates on the rebound principle, was scaled down and adapted to determine IOP of the mouse eye. IOP measurement using this concept is based on contacting the eye with a probe and detecting the motion as the probe collides with the eye and bounces back. The motion parameters of the probe vary according to eye pressure and are used to calculate IOP. A prototype instrument was constructed for measurement of IOP in mouse eyes, and its ability to accurately and reliably measure IOP was tested by comparing the measurements against the manometric (true) IOP determined in cannulated mouse eyes ex vivo. The I/I tonometer was also used to measure IOP in vivo in anesthetized adult C57BL/6 mice.

RESULTS. A strong correlation between the true IOP and the I/I measurements ($R^2 = 0.95$) was found for IOPs in the range of 3.7 to 44.1 mm Hg in cannulated mouse eyes. Repeat determinations in individual eyes showed a low degree of variability in the relationship of the measured IOP with the true IOP. In anesthetized mice, mean IOP ± SD as determined by rebound tonometry was 9.8 ± 3.9 mm Hg when the animals were anesthetized with ketamine alone and 7.6 ± 1.9 mm Hg when a mixture of ketamine, acepromazine, and xylazine was used. Contralateral eyes differed by 0.9 ± 2.5 and 0.1 ± 2.7 mm Hg, respectively, for the two anesthetic regimens.

CONCLUSIONS. The I/I tonometer can be used for noninvasive, in vivo IOP measurement in mouse eyes. The availability of an easy-to-use, reliable tonometer for IOP measurements in mice will allow more extensive use of the mouse as a model for glaucoma. (Invest Ophthalmol Vis Sci. 2003;44:1138–1141) DOI:10.1167/iovs.02-0553

Studies on the retinal pathology of glaucoma induced by elevated intraocular pressure (IOP) have advanced significantly thanks to the development of chronically elevated IOP glaucoma models in the rat.1–4 Although mouse strains with elevated IOP have also been identified,5–9 until recently, no method for noninvasive measurement of IOP in the mouse has been available. Previously, IOP measurements of the mouse eye relied on cannulation of the anterior chamber6–9 using microneedles. However, this procedure is technically demanding and difficult to learn. When a 50 μm diameter microneedle is used for cannulation,8 this method is successful approximately 80% of the time, can be used reliably only once per eye, and has significant risks of causing anterior chamber (AC) infection, AC inflammation, AC leaks, cataracts, and corneal scars.8 The use of still smaller diameter microneedles to minimize these risks, requires special physiological instrumentation not generally available in most laboratories involved in glaucoma research.9 In addition, both cannulation procedures can only be performed on fully anesthetized animals. These problems preclude use of this method in high-throughput studies or in repeated measurement and time-course studies of IOP in vivo.

Within the past year a noninvasive application method of measuring IOP in mice has been reported and used to measure IOP in both anesthetized and nonanesthetized animals.10–11 The modified applanation tonometer tip has a specially manufactured pair of prisms at a 60° angle10 and is used in conjunction with an appropriately modified tonometer. Although attractive in principle and fairly accurate in determining mouse IOP, this tonometer is hard to use (personal experience of JD). In addition, the subjective end point and the fact that application of the mouse eye affects IOP10 makes measurements more prone to bias. We set out to develop a noninvasive method of measuring IOP in mice that would avoid these potential problems and that would require minimal setup time per test, only a short training period for the user, and instrumentation that could be standardized across laboratories. Furthermore, we wanted a procedure that would be reliable, fast, and accurate, and could be performed on lightly anesthetized, or even on restrained but awake, animals. To achieve these goals we adapted the induction–impact (I/I) tonometer12–14 for use in mouse eyes.

This method is based on bringing a magnetized probe into contact with the eye and detecting its return-bounce motion with a sensing coil. The motion parameters of the probe vary according to eye pressure and can be used to determine the IOP. Significant potential advantages of this method over others include portability between different laboratories and users and the ability to obtain measurements in vivo without topical anesthesia.

The purpose of this study was to evaluate the applicability of the I/I method (rebound tonometry) for measuring IOP in the mouse.

MATERIALS AND METHODS

All experiments were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Tonometer

A schematic of the rebound tonometer is presented in Figure 1. Briefly, the tonometer consists of a pair of coils coaxial with the probe shaft that are used to propel the lightweight magnetized probe toward the cornea and to sense its movement. Appropriate electronic components

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allow for the probe movement to be initiated by the solenoid coil and monitored by the sensing coil. An applied pulse of electrical current induces a magnetic field within the solenoid, causing the probe to be propelled onto the cornea from where it rebounds. Motion parameters of the probe can be determined from movement of the magnetic probe, which generates a voltage in the sensing coil that is readily recorded and analyzed. The voltage is proportional to the magnetic field induced, which is proportional to the probe speed. Several motion parameters of the probe can be extracted from the sensing coil oscilloscope record and related to the IOP, such as the time of eye contact, the velocity of return, and the deceleration time. Previous work12–14 has established that the inverse of deceleration time (deceleration time–1) parameter is most closely correlated to IOP.

During preliminary testing of the instrument on enucleated mouse eyes, it was determined that a probe of 11 mg total mass with a shaft 24 mm long was optimal. The probe consists of a magnetized steel wire shaft with a round plastic tip (1 mm diameter) at its front end. This round tip minimizes the possibility of corneal damage from probe impact. Although generally more accurate, lighter probes are more prone to influence by external unrelated magnetic fields, which makes their behavior less predictable.

The tonometer solenoid and recording coils were custom made. The coils had resistances of 66 Ω (solenoid coil) and 205 Ω (sensing coil). All other I/I tonometer components except for the coils were made with standard commercially available electronic components. For detection of the probe movement, the signal from the sensing coil was amplified with an instrumentation amplifier (model INA121P; Texas Instruments, Dallas, TX). In the present experiments, the amplified sensing coil signal was recorded and displayed on a computer monitor for visual inspection with a virtual digital oscilloscope (Pico ADC-212; Picotechnology, Cambridgehire, UK) and saved for further analysis off-line. The speed immediately before impact, the deceleration during impact, and the ratio of these two parameters was determined from movement of the magnetic probe. Instead, the absolute voltage value at the time of impact and the slope of the voltage change after impact were the actual parameters used to determine this ratio. Both of these parameters are directly related to speed and deceleration, respectively, and yield a ratio that is proportional to the ratio of actual speed and deceleration of the probe.

**Device Calibration on Enucleated Eyes**

Eight mouse eyes (four from 3-month-old and four from 6-month-old animals) were obtained from terminally anesthetized young adult male C57BL/6 mice. Immediately after enucleation, the eyes were cannulated with a 26-gauge needle at 90° to the visual axis with the aid of an operating microscope. Cyanoacrylate glue was used around the point of entry of the needle in the anterior chamber to prevent leakage of aqueous humor. The absence of such leaks was verified microscopically throughout the experiment. IOP was controlled by adjusting the height of a variable column of balanced salt solution (BSS) attached to the cannula (open-stopcock method). IOP was verified and continuously recorded by a pressure transducer (Model TNF-R; Ohmeda, Louisville, CO) connected to the cannula, which was calibrated before eye cannulation by varying the height of the BSS column.

IOP was sequentially adjusted to 5-, 10-, 15-, 20-, 25-, 30-, 35-, 40-, 50-, and 60-cm water pressures (10 mm Hg = 13.6 cm water pressure). The eyes were suspended from the cannulating needle and rested against a vertical backing plate before the cornea being brought into contact with the tonometer probe from a horizontal direction. The tonometer probe and coil assembly were also positioned in a fixed horizontal position by an instrument clamp, so that the probe touched the cornea at its apex. Five measurements were made at each pressure level and the mean was calculated. The starting distance of the probe from the corneal surface was approximately 2.4 mm. Only measurements that occurred within a narrow time window (5 ms) 12 ms subsequent to triggering the tonometer probe were accepted for analysis. The ratio of probe speed immediately before impact over the deceleration during impact with the cornea was related to manometric (true) IOP. Therefore the ratio used for measurement, although given in units of time (seconds), was not derived from direct measurements of the time and distance of probe movement. Instead, the absolute voltage value at the time of impact and the slope of the voltage change after impact were the actual parameters used to determine this ratio. Both of these parameters are directly related to speed and deceleration, respectively, and yield a ratio that is proportional to the ratio of actual speed and deceleration of the probe.

**IOP Measurements In Vivo in Mouse Eyes**

Seven male C57BL/6 mice (6 months old) were used on two separate occasions. The animals were initially anesthetized with a mixture of 1 mL/kg xylazine-ketamine-acetpromazine (9, 45, and 1 mg/mL, respectively) and IOP was measured by the I/I method. The animals were then allowed to recover. One week later, IOP measurements were repeated after the animals were anesthetized with ketamine only (250 mg/kg). Five tonometer measurements were obtained on each occasion in each eye within 5 minutes from the time point when the animal became unresponsive to deep pressure sensation. From these measurements, the average IOP was calculated from the calibration formula (Fig. 2).

**Statistical Methods**

Statistical analysis was performed on computer (Excel; Microsoft Corp., Redmond, WA; and NCSS software; NCSS, Kaysville, UT). Linear regression was used to calculate correlation coefficients. Multiple re-
gression with dummy variables was used to compare the slopes of multiple-regression lines. A paired t-test was used to detect intereye differences, and the unpaired t-test was used to compare the mean IOP of mice under the two different anesthetic regimens.

RESULTS
Device Calibration
Graphs of the correlation between manometrically determined (true) IOP and I/I tonometer readings are shown in Figure 2. There is good linear correlation between log of true IOP and the tonometer readings ($R^2 = 0.95, P < 0.0001$). Multiple regression analysis showed that sequential replicate measurements at any one pressure level for each eye differed to a small, but significant, extent ($P < 0.001$). This variability accounts for a change in the $R^2$ of only 0.005 ($R^2 = 0.956$ vs. 0.959 when the full model is used). Calibration curves of individual eyes were found to differ by a small but significant degree ($P < 0.001$). This variability was not dependent on the age of the animal ($P > 0.8$). Inspection of the data from individual eyes showed that the variability was primarily due to a variation of a single eye within the set that accounted for a change in the $R^2$ of only 0.005 ($R^2 = 0.954$ vs. 0.959 when the full model is used). This minimal effect of biological variability justifies pooling of the measurements from all the eyes to generate a "master" calibration curve for the particular instrument and strain of mouse that was subsequently used to determine in vivo IOP from tonometer readings.

IOP Measurements In Vivo in Mouse Eyes
Normal mouse IOP was found to be 9.8 ± 3.9 mm Hg (mean ± SD) when ketamine alone was used and 7.6 ± 1.9 mm Hg when ketamine-xylazine-acepromazine was used for anesthesia. IOP under the anesthetic mixture was thus lower than IOP under ketamine only anesthesia ($P = 0.034$ one-tailed t-test). Differences between contralateral eyes was 0.9 ± 2.5 mm Hg under ketamine and 0.1 ± 2.7 mm Hg under ketamine-xylazine-acepromazine. IOP of contralateral eyes did not differ significantly among animals under either anesthetic regimen ($P > 0.2$ and $P > 0.4$ respectively).

DISCUSSION
Rat models of glaucoma have gained popularity over the past 10 years, contributing significantly to our current understanding of the pathophysiologic mechanisms involved in the disease at the cellular and molecular levels. However, rat models of glaucoma do not afford the opportunity to use the extensive genetic information relating to glaucoma that has been and continues to be collected. Use of such information would be greatly facilitated if mouse models of glaucoma were available. To date, three related mouse strains have been reported that develop a spontaneous form of a secondary angle-closure glaucoma. The disease in these animals shows significant similarities with, as well as some important differences from, the human disease.

A significant impediment to working with these models has, until recently, been the inability to measure IOP noninvasively in vivo. The introduction of a modified applanation tonometer for in vivo measurements of IOP is a start toward solving this problem. However, the applanation method has significant shortcomings. Most notably, the process of measuring IOP in the mouse affects IOP itself. Cohan and Bohr noted that at least six successive measurements are needed before IOP stabilizes at a lower point. Although not discussed by the investigators, the volume of aqueous displaced to applanate the mouse eye with the modified tonometer prism is approximately 3% (assuming an aqueous volume of 5 μL). This introduces a significant change from the initial IOP if applanations are repeated six times. In addition, the subjective end point of this method (aligning two hemispheres) depends on the amount of fluorescein applied to the eye, which is a poorly controlled variable. Finally, although most mouse strains have protruding eyes, it is not always easy to correctly applanate an area of the mouse cornea with a diameter as large as 1.5 mm for an eye only 3 mm in diameter.

It would thus be a desirable improvement to have a method for IOP measurement in mice that is repeatable, rapid, and reliable and that is operator independent and consistent for measurements done over extended periods of time. We report the development of such a method.

The principle of rebound tonometry has been described. It is closely related to the principle of vibration tonometry and the earlier Krakau tonometer. It has been applied recently for measuring IOP in large animal eyes ex vivo and clinically in humans. It has also been successfully adapted for use on rat eyes. The modifications needed to use the method for the smaller mouse eye are relatively minor. We constructed a prototype instrument to allow us to test the correlation of true (manometric) IOP and tonometric determinations by using the rebound principle. The results indicate that after calibration in ex vivo eyes, the I/I tonometer can be used to determine the true IOP of mouse eyes in vivo.

Variability of readings for IOP in each eye tested ex vivo, as well as among replicate measurements, was found to be very small, indicating good reproducibility of the instrument measurements. Age did not appear to affect IOP measurements at least in the two adult age groups tested (3 and 6 months). Such a lack of dependence on age would be advantageous if individual animals are to be observed for many months.

The effect of the starting distance between the probe and cornea was not investigated, because it was found that it is fairly easy to keep this distance relatively constant. However, similar measurements made in the rat eye appear to be relatively unaffected within a range of initial distance of the probe from the corneal apex (between 3 and 5 mm) before its launch and also for angles of impact of the probe with the corneal apex relative to the visual axis of less than 25°.

Visual inspection of the oscilloscope tracing that records the sensing coil current, allows for rejection of the occasional signal that does not exhibit the appropriate waveform for IOP estimation. However, this occurs infrequently, and we found that the instrument software was reliable in rejecting these signals. Although the IOP measurements with the prototype instrument reported herein were performed off-line, software to allow on-line measurements is feasible and is currently being developed and tested.

Measurement of IOP in live animals is the real test for any tonometric method. In the experiments outlined earlier, IOP in live C57BL/6 mice measured with the I/I tonometer had a good agreement with IOPs found by cannulation in normal mice (10–16.5 mm Hg). In addition, IOP did not differ significantly between contralateral eyes when measured with the I/I tonometer. Ketamine anesthesia seemed to decrease IOP less than the ketamine-xylazine-acepromazine mixture, as has previously been reported.

The successful use of the I/I method of tonometry in mice allows the planning of longitudinal studies of IOP measure-
ments in other strains and phenotype determination in mice with glaucoma-related transgenes or mutations, potentially enhancing our understanding of the disease.

References


