Assessing Intraocular Pressure by Rebound Tonometer in Rats with an Air-Filled Anterior Chamber

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Abstract

Purpose: To compare rebound tonometer and cannulation as methods for measuring intraocular pressure (IOP) in rats.

Methods: The accuracy of the TonoLab rebound tonometer was determined in eight cannulated rat eyes. IOP was manipulated by changing air pressure from 20 to 100 mmHg at 10-mmHg intervals, and the IOP was measured with the rebound tonometer at each level. The average value of three repeated pressure readings was recorded. Correlation analysis and comparison with the Bland and Altman method were performed. The intraclass correlation coefficient was calculated to assess intraoperator variability.

Results: The IOP values measured with the TonoLab rebound tonometer were well correlated with the actual IOP ($r^2 = 0.963, P = 0.01$). The mean of the difference between the rebound tonometer and actual (cannulation) IOP was $7.41 \pm 7.87\%$. The intraclass correlation coefficient was 0.9, indicating low intraoperator variability.


Key Words: cannulation, IOP measurement, rat, rebound tonometer

Introduction

Murine and human eyes share a number of anatomic and physiological similarities, including well-established uveoscleral and trabecular meshwork outflow pathways and similar IOP responses to topical glaucoma medication. Furthermore, rodent IOP is comparable to human IOP and is relatively easy to manipulate. These features make the murine system particularly attractive as animal models for glaucoma.

Cannulation of the anterior chamber permits measurement of true manometric IOP in the mouse. However, this procedure is technically difficult and necessitates perforation of the cornea and administration of general anesthesia. These disadvantages have led to the development of noninvasive techniques for IOP measurement in the murine glaucoma model.

Because of its easy handling and portability, the Tono-Pen XL tonometer (Medtronic, Jacksonville, FL, USA) is currently the most widely used device for measuring rat IOP. However, as it was designed for the human eye, the probe tip is large relative to the rat eye and it has a tendency to underestimate IOP. The Tono-Pen’s reliability for mea-
measurements in rat eyes depends on the direction of the probe and the pressure of contact, both of which can vary with the experience of the operator.

The rebound tonometer is based on the rebound principle—bouncing a magnetic probe onto the eye and using its motion parameters to determine IOP. The probe is small and lightweight, and it produces a lower stimulus, eliminating the need for corneal anesthesia.1 There are several studies comparing the anterior chamber cannulation method and the rebound tonometer, most of which manipulate IOP by hydraulic pressure through the use of a balanced salt solution.1,2 To our knowledge, there has been no study of this tonometer using barometric pressure to manipulate IOP. However, in ischemia reperfusion models, barometric pressure has been widely used for manipulation of rodent IOP.3 In the present study, we compared IOP value results obtained by the cannulation method and the rebound tonometer method in rat eyes filled with air at various IOPs, manipulated by barometric pressure.

Materials and Methods

The subjects of this study were eight eyes of eight male albino Sprague-Dawley rats (250–300 g, 8 weeks old). The rats were housed in transparent plastic rodent boxes under a 12-h light–dark cycle, with lights on starting at 6 a.m. Food and water were available ad libitum.

Baseline IOP before anesthesia was measured using the TonoLab rebound tonometer (Colonial Medical Supply, Franconia, NH, USA) following the manufacturer’s recommended procedures. The mean of three valid IOP readings obtained from six IOP measurements was obtained from each experimental and fellow (control) eye. All animals were acclimated to daily handling for 1 week, and normal awake IOPs were confirmed before anterior chamber cannulation.

The rats were anesthetized through intramuscular injection of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg), followed by topical proparacaine hydrochloride 0.5% eyedrops (Alcaine, Alcon Laboratories, Fort Worth, TX, USA). Measurements were initiated as soon as the mouse was sufficiently anesthetized, indicated by failure of the mouse to respond to a foot pinch.

Cannulation of the anterior chamber was performed with a 30-gauge needle attached via polyethylene tubing to a three-way connector, which in turn was connected parallel to a pneumatic pressure device (open-stopcock method). The needle tip was fixed and placed within the anterior chamber while avoiding contact with other ocular structures: corneal endothelium, iris, lens, or retina. The eyes were placed on a platform of adjustable height, with the eye adjacent to the tonometer tip. The intracameral pressure was adjusted from 20 to 100 mmHg at 10-mmHg intervals. At each level, an experienced optometrist evaluated IOP with the rebound tonometer. Measurements were carried out through careful operation of the measurement button, while the tonometer was maintained in a stable position, with the probe set perpendicular to the ground and to the corneal surface and the tip of the probe directed to hit the central cornea. The starting distance of the probe from the corneal surface was 3–5 mm. A new disposable probe tip was used for each subject. Six measurements were taken consecutively. After the sixth measurement, the letter P appeared in the display, followed by the IOP reading. Three series of six measurements were obtained, and the average value was recorded.

All statistical tests were performed by computer (SPSS 13.0 software for Windows; SPSS, Chicago, IL, USA). Pearson correlation analysis was performed to evaluate the correlation between the true (cannulation) IOP and the IOP reading by rebound tonometer, and intraoperator variability was established by calculating intraclass correlation coefficients. A Bland–Altman plot of the difference between rebound tonometer and cannulation IOP readings against the average of the two was constructed, with the intent of assessing the agreement between the two methods and the presence of systemic bias.

The mean IOP for the eight eyes was 11.4 ± 2.12 mmHg (range, 8.7–15 mmHg) as measured by rebound tonometer. The corresponding linear regression describing the relationship of the TonoLab IOP measurements with true IOP was $y = 0.753x + 7.429$ ($r^2 = 0.963; P = 0.01$; Fig. 1).

A Bland–Altman plot showed good comparability in that most of the values were within the range of ±1.96 SD from the average [cannulation IOP − Rebound IOP] value. The plot also revealed a systematic proportional bias, as IOPs measured with the rebound tonometer were consistently lower (7.41 ± 7.87%) than those recorded with the cannulation technique, and this difference increased for

![Figure 1. Scatter plot showing the correlation between the measured intraocular pressure (IOP) and the actual IOP. Rebound tonometer values are plotted against cannulation values. The regression line formula is $y = 0.753x + 7.429$ ($r^2 = 0.963$).](image-url)