EVALUATION OF PERKIN’S APPLANATION TONOMETER AND THE NORMAL RANGE OF INTRAOCULAR PRESSURE IN ANESTHETIZED RATS

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ABSTRACT—To assess the reliability of noninvasive measurement of intraocular pressure (IOP) in rats, a Perkin’s applanation tonometer was calibrated against direct manometry. The normal values of IOP in male Wistar rats were then detected. The mean tonometer readings against the transducer IOP produced regression formula: \( y = -0.198 + 1.071x \) \( r^2 = 0.987 \). The mean IOP with standard deviation in rats was 17.7 ± 3.5 mm Hg (95% and 99% confidence intervals: 17.2 and 18.1 mm Hg, 17.0 and 18.3 mm Hg for the lower and upper limits of the normal rat IOP, respectively). The IOP could be measured accurately using Perkin’s applanation tonometer in anesthetized rats each weighing 300 g and over. Measurement of IOP using this tonometer was considered to be valuable allowing, repeated use in rats because of its small size, portability and noninvasiveness.

KEY WORDS: Applanation tonometer, IOP, Rat

INTRODUCTION

Applanation tonometers estimate the intraocular pressure (IOP) by measuring the force required to flatten a constant area of the central corneal surface. The tonometers have been considered to be highly reliable for measuring IOP clinically or experimentally in dogs, cats, monkeys and rabbits but not in rodents because of the small size of the applanate region in the corneal surface (Hammond and Bhattacherjee, 1984; Priehs et al., 1990; Walker et al., 1972). Since various animal species are used in the ophthalmic studies, it is very important to establish normal variations in the IOP of such small laboratory animals as rats and mice. Rodents are preferred species, as they are less expensive, the experimental conditions are much more easily controlled. Perkin’s applanation tonometer has been suggested to possess the advantages of availability for rodents, lower cost, easy operation and portable size (Niwa et al., 1995). The Goldmann prism in this tonometer approximates a region of the cornea 3.06 mm in diameter which induces a very small displacement of aqueous humour and a negligible IOP increment in humans (Mermoud et al., 1994). There are numerous investigations of the drug-related IOP changes, e.g., corticosteroids (Rohen et al., 1973; Spaeth et al., 1977), adrenergic drugs (Neetens and Bernard, 1973), anesthetic (Bar-Ilan and Pessah, 1986) and decongestive agent (Innemee and Zwieten, 1978). Therefore, the tox-
icologists and/or ophthalmologists, by virtue of their specialized techniques and methods, must contribute the informations to clinical effects on the eye. In the present study, after calibration, i.e., IOP measured by the use of a Perkin's applanation tonometer was compared to that of direct manometry and then IOP in rats was measured noninvasively, and the normal value was determined.

MATERIALS AND METHODS

A total of 120 male Wistar rats (CLEA Japan Inc., Japan) each weighing 300 to 400 g were used. At the start of the experiment, the animals were 20 weeks old. They were housed individually in a stainless steel cage in a clean cabinet with the following conditions: temperature, 20 to 26°C; relative humidity, 40 to 70%; air exchange, 8 to 12 cycles/hr; and lighting, 12 hr light-dark cycle (light on from 8:00 to 20:00) and fed laboratory chow ad lib. Maintenance and experimental conditions conformed to the Guide for the Care and Use of Laboratory Animals of Takeda Chemical Industries, Ltd.

Before calibration, all animals were anesthetized with ketamine hydrochloride (100 mg/kg, i.m.) and droperidole hydrochloride (1 mg/kg, i.m.). The rats were then positioned in ventral recumbency after the instillation of a local anesthetic for ophthalmic examination, oxybuprocaine hydrochloride (0.01 μg/drop), which was dissolved in physiological saline to make a 0.025% (W/V) solution. The 26-gauge needle was inserted into the anterior chamber of 40 normal eyes of 20 Wistar rats temporally through the cornea at a flat angle because the iris protruded hemispherically into the anterior chamber. During cannulation, there was no leakage of aqueous humor around the needle. Care was taken not to touch the long posterior ciliary artery. The needle was connected via a polyethylene tube to a micro-injection syringe with physiological saline and an electronic pressure transducer (DX-360, Nihon Kohden Ltd.). Pressure was then amplified with an amplifier (AP-64/G, Nihon Kohden Ltd.). In this condition, IOP could be adjusted by adding or removing a physiological saline of the syringe to the anterior chamber. Intraocular pressure was determined in millimeters of mercury on the recording chart and the display of an amplifier. At the start of measurement, the recorder and amplifier were calibrated against the mercury column. The rat cornea was then stained with a fluorescein indicator (FLUORES Test Paper, Showa Pharmaceutical Ltd.). Then, IOP was adjusted transiently 0 mm Hg by removing the aqueous humor from the anterior chamber. Intraocular pressure was then increased by infusing saline increment of 1 μl into the anterior chamber. After stabilization for 1 to 2 minutes at each IOP level, IOP with a Perkin's hand-held applanation tonometer (Clement Clarke Ltd., London) was measured. When the prism of a Perkin's applanation tonometer properly applanated the corneal surface of the rat's eye, the inside edge of the fluorescein ring was connected to that of another ring (Fig. 1). Furthermore, best fluorescein ring for rat was considered that each inside edge was set close to each outside edge. Three readings using the tonometer were recorded at each IOP level measured by the pressure transducer. The corneal surface was moistened properly with physiological saline during the measurement. Figure 2 shows the procedure for noninvasive IOP measurement using Perkin's applanation tonometer.

RESULTS AND DISCUSSION

The mean of 3 measurements each for calibration of Perkin's applanation tonometer was plotted against corresponding manometric values. Linear and polynomial regression analyses were performed to obtain the mathematical formulation of the relationship between each tonometric measurement and the manometric value. The mean tonometer values for 40 eyes of 20 rats against the transducer IOP produced formula: \( y = -0.198 + 1.071x \quad (r^2 = 0.987) \) (Fig. 3). Furthermore, to determine the normal IOP in 200 eyes of 100 other Wistar rats, IOP was measured with a Perkin's tonometer. The mean with standard deviation was calculated, and 95% and 99% confidence intervals were also calculated to certify the normal IOP for Wistar rats. The IOP of the right and left eyes were similar (17.6 ± 3.3 mm Hg and 17.7 ± 3.6 mm Hg for the mean IOP, respectively; \( P = 0.01 \), Student's paired t-test). The mean IOP in 200 eyes of 100 rats using a
Noninvasive measurements of intraocular pressure (IOP) in Rats

Fig. 1. Fluorescein rings through the finder. Right figure shows the prism applanating properly an area of the corneal surface, whereas the central and left figures denote the prism applanated lightly and too lightly, respectively.

SYSTEMIC ANESTHESIA

INSTILLATION OF A LOCAL ANESTHETIC IN VENTRAL RECUMBENCY

CORNEA STAINED WITH FLUORESCINE

LIGHT CONTACT OF THE PRISM TO CORNEA

GRADUAL APPLANATION

DETECTION OF THE BEST FLUORESCINE RING

IOP READING

Fig. 2. Procedure for noninvasive IOP measurement.

The tonometer was 17.7 ± 3.5 mm Hg. IOP using the above mentioned formula (normal corrected IOP) was 16.7 ± 3.1 mm Hg. The confidence interval of 95% and 99% was 17.2 and 18.1 mm Hg, 17.0 and 18.3 mmHg for the lower and upper limits of normal IOP, respectively.

The normal corrected IOP, 16.7 ± 3.1 mm Hg, is higher than that reported by Funk et al. (1985), who detected that the normal IOP measured by direct manometry was 15.9 ± 0.4 mm Hg in Wistar Kyoto rats under anesthetic conditions. Recently, Mermoud et al. (1994) evaluated the Tono-pen tonometer for measuring IOP in the anesthetized Lewis rats (weighing 150 to 200 g) and reported that the normal corrected IOP was 17.30 ± 5.25 mm Hg. This difference may be due to differences in the anesthetics used, the strain of rat, the posture of animals or the apparatus for measurement. Bar-Ilan et al. (1986) demonstrated that 30 to 90 mg/kg of ketamine showed an increase in rabbit IOP. Therefore, great care should be taken in the interpretation and comparison of ocular pharmacological data obtained in ketamine-anesthetized animals (Antal et al., 1978; Schutter and van Horn, 1977). Although all the measurements in this study were performed rapidly within 10 min.

Fig. 3. Relationship of Perkins tonometer measurements of IOP to the closed manometric pressure measurements (pressure range, 2.8 to 48.0 mm Hg). The solid line is the calculated regression. The dotted line is the ideal linear model.
after ketamine-anesthesia, ketamine might affect slightly the IOP. Also, IOP showed a large variation by the posture of the animal (Leonard et al., 1983). Therefore, the animal should be kept in the same position during the measurement. In the present study, the operation was easy with the rat positioned in the ventral recumbency and IOP could be measured stably. Moreover, the present data obtained from the experiment for calibration of Perkin’s applanation tonometer showed that goodness of fit in 30 mm Hg or less was detected ($r^2=0.987$) but the tonometer overestimated pressures higher than 30 mm Hg. Although we cannot explain this phenomenon, it might be due to leakage of aqueous humor around the needle at the higher pressure range. The difference of an apparatus or methods may result in the difference regarding the slope or y-axis of the regression line. In addition, noninvasive IOP measurement using a Perkin’s applanation tonometer also has the disadvantage of difficulty of measurements using small rats and estimating IOP after keratoplasty, the presence of corneal edema and other corneal abnormalities in our preliminary rat studies. Especially, the intraocular pressure measurements using this tonometer in smaller rats should be excluded from analysis because of the lack of reproducibility for small size of an applanate region in the corneal surface.

Except for the selection of anesthetics and the limitation of rat body weight, IOP using a Perkin’s applanation tonometer could be measured accurately in anesthetized rats. Measurement using this tonometer was considered to be valuable allowing, repeated use in rats because of its small size, portability and noninvasiveness.

REFERENCES


