Experimental Eye Research 121 (2014) 86–93

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Validation of rebound tonometry for intraocular pressure measurement in the rabbit

Hong Zhang [a,b,*], Dong Yang [a], Craig M. Ross [a], Jonathan P. Wigg [a], Surinder Pandav [a,c], Jonathan G. Crowston [a]

[a] Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, 32 Gisborne Street, East Melbourne, VIC 3002, Australia
[b] Eye Hospital, Harbin Medical University, No. 157 Baojian Road, Nangang District, Harbin, Heilongjiang Province 150001, China
[c] Postgraduate Institute of Medical Education & Research, Sector 24-A, Chandigarh 160012, India

ARTICLE INFO

Article history:
Received 11 September 2013
Accepted in revised form 7 February 2014
Available online 20 February 2014

Keywords:
glaucoma
intraocular pressure
rabbits
tonometer
non-invasive

ABSTRACT

Rabbits play a growing role in research into glaucoma surgical models and ocular drug delivery models. However, the lack of an accurate method for measuring intraocular pressure (IOP) in this animal has been a significant deficit. In this study we validated the use of the TonoVet rebound tonometer and provide conversion tables for its use in rabbits. Experiments were performed on 18 adult New Zealand White rabbits. The TonoVet measurements were obtained and compared to manometric readings by anterior chamber (AC) cannulation. The TonoVet position and 'd' (dog or cat) and 'p' (other species) modes were compared. The sensitivity of the TonoVet tonometer in assessing IOP changes was also tested. There was a strong linear correlation for both the 'd' mode (mean slope = 0.84 ± 0.03, $r(2) = 0.99 \pm 0.03$) and the 'p' mode (mean slope = 0.64 ± 0.02, $r(2) = 0.97 \pm 0.01$) of the TonoVet with manometric IOP. However, the TonoVet had a tendency to underestimate IOP compared to manometry and conversion formulae were possible to calculate for both modes. The orientation of the TonoVet handle had no effect on IOP reading, as long as the groove was horizontal. No significant differences were observed when comparing right and left eyes ($P > 0.05$). IOP recovered four days after cannulation. Younger rabbits had lower IOP compared with older rabbits ($P < 0.01$). Timolol produced a 2.5 mmHg reduction in IOP 2 h later as detected by the TonoVet. Using the conversion table presented, the TonoVet is a reliable and precise tool for the measurement of IOP in rabbits.

1. Introduction

The use of rabbit experimental models in glaucoma research is well established, and much is known about the anatomy and physiology of the rabbit eye. Rabbit eyes are not dissimilar to human eyes in size, offering advantages in testing certain drugs, surgical procedures and new treatment modalities (Alvarez et al., 2013; Bourges et al., 2006; Chang et al., 1998; Hurvitz et al., 1991; Lavery and Kiel, 2013; Lim et al., 1998; Lloyd et al., 1996; Melamed et al., 2003; Nguyen et al., 2012; Percicot et al., 1996). Reducing IOP is currently the mainstay of glaucoma treatment. Therefore, an accurate, reproducible, and convenient method of IOP assessment for the rabbit is of central importance.

Live manometry is an invasive, yet precise method of measuring IOP, and is the gold standard by which all other tonometers are compared (Kniestedt et al., 2008). However, this procedure is technically demanding and difficult to perform. It 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 (Best et al., 1970; Blumenthal et al., 1992; Morris et al., 2006). Further, it necessitates perforation of the cornea and the administration of general anaesthesia; both of these may affect IOP. In addition, several days are required for the corneal perforation to heal before a subsequent IOP measurement can be reliably obtained. These disadvantages have led to the development of non-invasive techniques for IOP measurement.

The current non-invasive IOP measurement devices used in rabbits include the Tonopen XL, the Perkins handheld applanation tonometer and pneumatonometry. Both Lim et al. (2005) and Abrams et al. (1996) concluded that the pneumatonometer was the least-accurate method of measurement and had the most variability. The Tonopen XL and Perkins tonometers have been shown to underestimate IOP, with increasing inaccuracy at higher

* Corresponding author. Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, 32 Gisborne Street, East Melbourne, VIC 3002, Australia; Eye Hospital, Harbin Medical University, No. 157 Baojian Road, Nangang District, Harbin, Heilongjiang Province 150001, China. Tel.: +61 3 9288 3721; fax: +61 9662 3859.
E-mail addresses: hong.zhang@unimelb.edu.au, dr.hzhang2007@hotmail.com (H. Zhang).

http://dx.doi.org/10.1016/j.exer.2014.02.004
0014-4835/

pressures. The Tonopen XL was the most accurate at predicting true IOP; but the variability of this instrument was its major drawback. The Perkins applanation tonometer had significantly less variability of measurement; however, it is not as accurate overall (Abrams et al., 1996; Lim et al., 2005). None of the tonometers are accurate or reproducible in estimating IOP in rabbits over the tested range.

The rebound tonometer (originally called an induction/impact tonometer), which has been suggested recently for non-invasive IOP measurement, was first introduced in 1931 (Munkwitz et al., 2008). Utility for this device has been validated for use in mice (Morris et al., 2006; Wang et al., 2005) and monkeys (Yu et al., 2009) but not previously in rabbits. Rebound tonometry uses a lightweight magnetic probe that is propelled by a solenoid from the unit and aimed at the centre of the cornea. The deceleration of the probe during the impact, which correlates to IOP, can be monitored by the voltage change induced by the moving magnetic probe. A globe with low IOP will have a longer impact time and slower deceleration; and a globe with high IOP will have a shorter impact time and faster deceleration. Recently, two hand-held apparatuses based on this technical principle became commercially available for animal studies: the TonoLab rebound tonometer intended for use in rodents and the TonoVet tonometer for use in larger animals.

The TonoVet has 3 operating modes – 'h', 'd' and 'p' for horses, dogs/cats and other species, respectively. It is not known which mode is best for IOP measurement in the rabbit. Pereira et al. (2011) compared the TonoVet and the Tono-Pen in normal rabbits and showed the linear relationship between the two tonometers. However, IOP measurements may vary depending on the instrument model and the operating mode used. Moreover, due to the co-varying and confounding errors of each tonometer, the accuracy of a tonometer can only be determined by comparing it to simultaneous live manometry.

Only one study by Kalesnykas et al. compared live manometry with the TonoVet, Perkins handheld and Tono-Pen XL. They reported a tendency for the TonoVet to underestimate IOP and that the Tono-Pen XL has higher deviation than the Perkins and the TonoVet (Kalesnykas and Uusitalo, 2007). Perhaps due to the small number of rabbits ($n = 2$) tested in this study, each 5 mmHg increase in manometric IOP resulted in varying changes to the TonoVet readings, with no discernible pattern.

In the current study, we aimed to evaluate the accuracy and precision of the TonoVet compared to manometrically set IOP, in a larger group of rabbits and with a view to developing a conversion table to enable use of the TonoVet in future research.

2. Methods

2.1. Animals and anaesthesia

In this study, 18 female New Zealand White (NZW) rabbits were used. Among them were 12 rabbits with an average age of six months and mean weight of 3.5 kg; and 6 rabbits with an average age of three months and mean weight of 2.9 kg. Animal experiments were conducted with the approval of the Royal Victorian Eye and Ear Hospital (RVEEH) Animal Research & Ethics Committee (AREC) and were consistent with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. The animals were housed under a 12-h/12-h light/dark cycle with lights on starting at 6:00 AM; animals used for drug treatment were entrained to a lighting schedule of alternating 12 h periods of light and dark for at least 3 weeks prior to experiments. Food and water were available ad libitum.

General anaesthesia was performed for the cannulation procedure. Rabbits were anaesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg) administered intramuscularly. Topical instillation of 0.5% w/v oxybupivacaine drops were applied as required for anaesthesia of the ocular surface and to ensure that eyes remained moist. For daily IOP measurement with the TonoVet only, no anaesthesia was needed.

2.2. Methods for rebound tonometry

The TonoVet was used according to the manufacturer's instructions, with the horizontal position of the magnetic probe and the tonometer tip perpendicularly directed from 6 mm away to the central cornea in both positions. To minimize the potential for operator-introduced bias, a spirit-level was used to maintain the horizontal position of the magnetic probe, and a ruler used to standardize the measurement distance (Fig. 1). The calibration was done with both 'd' and 'p' modes. Only the readings considered acceptable by the TonoVet (that is, within the range of acceptable standard deviation as indicated by no bars or one bar on the instrument display) were reported. The equipment was programmed to average the IOP values of six consecutive, acceptable measurements and produce a reading of the mean IOP. In the subsequent parts of this study, three readings (each a mean of six measurements; a total of 18 separate measurements) were obtained under each condition.

For daily IOP measurement in waking rabbits, the TonoVet may be held in the same fashion as rebound tonometry in humans, with the device handle vertically below the probe. This is hard in anaesthetized rabbits as there is not enough space between the eye and supporting table. However, it is also possible to hold the device handle rotated 90° with the handle at the same horizontal height as the probe (Fig. 1). To test whether the position of the TonoVet would affect the IOP measurement, measurements were taken with the handle held both horizontally and vertically.

2.3. Cannulation technique

Manometric IOP measurement was performed using a syringe pump and pressure transducer apparatus from Harvard Apparatus. A P75 venous pressure transducer was coupled via an amplifier (Harvard Apparatus TAM-A with 0–10 V DC output) to a PHD ULTRA CP constant pressure syringe pump). A 10 mL Terumo plastic syringe was mounted in the syringe pump and connected via sterile tubing to the pressure transducer and a 26-gauge needle. The system was filled with sterile Hanks' balanced saline solution (BSS). The pressure transducer was rinsed with 70% ethanol followed by several flushes of sterile BSS before use. The 26-gauge needle was used for anterior chamber cannulation and was situated at the distal end of a 50 cm length of tubing from the pump & transducer apparatus. Prior to anterior chamber cannulation the transducer was set to zero pressure at eye level with the needle tip in fluid, and the transducer calibration verified by movement of the needle tip compared to a ruler height corresponding to a fluid pressure change of 30 mmHg (1 mmHg = 1.36 cmH_2O).

2.4. Calibration of the TonoVet

Calibrations were performed in the right eye of 6 six-month-old NZW rabbits. After anaesthesia, rabbits were placed recumbent in a prone position. Eyelids were opened with a speculum and the 26-gauge needle which was attached to the cannulation system was inserted through the peripheral cornea. Care was taken to ensure that the resting globe position was the same as that prior to needle entry; in addition, the needle was positioned to ensure no detectable change to the corneal curvature.