



Measurement of Intraocular Pressure in a Porcine Ex Vivo Model Eye

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Authors' contributions

This work was carried out in collaboration between all authors. Author IS designed the study, managed the literature searches, wrote the protocol, and wrote the first draft of the manuscript. Authors RM and FU managed the analyses of the study. Author RM performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To compare the pressure inside and outside the pig eye in relation to the location of cannulation for the injection of liquid into the anterior or vitreous chamber.

Method: Eleven enucleated pig eyes were used. 46 measurements of intraocular pressure IOP were taken with a Perkins tonometer when the eye was cannulated in the anterior chamber. 49 measurements were taken when the eye was cannulated in the vitreous chamber. The eyeball was connected to a low-pressure transducer to control, maintain and modify the pressure in the eyeball.

Results: No significant difference ($P=0.138$) was found between the Perkins pressure measurements from cannulations in the anterior and vitreous chambers. A linear relationship between transducer and Perkins measurements was found when the eye was cannulated in the anterior chamber ($IOP=-7.749+0.763$ transducer; $R^2=0.940$, $p<0.001$) and when it was cannulated in the vitreous chamber ($IOP=-7.476+0.730$ transducer, $R^2=0.885$, $p<0.001$). No difference was found between the Perkins/transducer pressure ratios ($P=0.500$ ANOVA) from cannulations in anterior and vitreous chambers. There were no differences in the measurements among eyes that could affect pressure outcomes. A direct relationship between the insufflate pressure inside the eyeball and the Perkins

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pressure was found.

Conclusion: The pressure measured by Perkins applanation tonometry in an ex vivo porcine eye model is not correlated with the area of cannulation, keeping constant pressure with a low-pressure transducer. A linear equation was generated that correlates the pressure gauge with IOP Perkins, which would apply to future studies that use the pig eye as an ex vivo animal model of hypertension in glaucoma.

Keywords: Ex vivo animal model; glaucoma model; hypertension model; pig eye animal model.

1. INTRODUCTION

Glaucoma is pathology with a neurodegenerative etiology. It is irreversible and is the second leading cause of blindness worldwide [1]. The prevalence is between 1% and 3%, depending on the study population [2,3], and around 50% of glaucoma patients remain undiagnosed [4]. Glaucoma is characterized by a loss of ganglion cells in the retina and is conditioned by genetic factors [5]. This loss is mainly caused by increases in the intraocular pressure (IOP). The maintenance of IOP above 21 mm Hg causes a thinning of the lamina cribosa [6], an area through which the ganglion cells of the retina must pass. The death of nerve cells results in permanent defects in the visual field [2,7,8].

The porcine eye is a commonly used glaucoma animal model because its size is similar to the human eye, and it has been used as an ocular hypertension model or glaucoma model [9-19]. There are different methods for generating an ocular hypertension model: cauterizing episcleral veins [9,10], blocking the iridocorneal angle to prevent aqueous flow through the trabecular meshwork [11], injecting fluids into the eye in live animals [10,12] and developing ex vivo models [13,14,20].

These animal models are used for pharmacokinetic and pharmacodynamic studies testing new treatments for reducing IOP in glaucoma [15,16,21], developing laser treatments with similar function to the iridotomy [18], monitoring the loss of ganglion cells [9,11] and introducing lesions in the lamina cribosa [12,13]. In ex vivo models of ocular hypertension with injection of liquid, the best method for liquid infusion and their effects on IOP measurements are not clear. Some studies have performed cannulation in the anterior chamber [17,19], others in the vitreous chamber [22] and some through the optic nerve [23]. However, there are no studies comparing the changes in IOP from infusions in different areas of the eye. Usually, Goldman tonometry is not used on animals because it requires a slit lamp to conduct the measurements. For this reason, Perkins applanation tonometry or other tonometers, such as a Tonopen [24,25], are used frequently.

The purpose of this study was to compare two different methods of increasing IOP in a porcine eye ex vivo model, anterior chamber infusion versus vitreous chamber infusion, to determine whether these induce different IOPs and how they affect the experimental model.

2. MATERIALS AND METHODS

The study was conducted in IOBA Eye Institute, University of Valladolid (Spain). Eleven enucleated pig eyes (*Sus scrofa domestica*) were obtained from the local abattoir. Animals were white (not albino) domestic pigs between six and eight months of age, and they

weighed 120 to 150 kg. The eyes were enucleated around 8:30 AM, just after slaughter. The measurements were made between 9:00 AM and 12:00 AM. Excess tissue surrounding the eyeball was removed with Moorfield conjunctival forceps and Wescott tenotomy wide-handle scissors (John Weiss international, Milton Keynes, UK) to facilitate the experimental measurements.

An experienced surgeon did not perform enucleation. For this reason, eyes with signs of any trauma, including loss of form, visible retinal detachment, lens dislocation and other, which were verified via direct ophthalmoscopy before performing the experimental measurements, were not used for this study.

2.1 Anterior Chamber Infusion

Forty-six Perkins measurements were taken from six porcine eyes that were cannulated with a 23-G cannula, in the anterior chamber at 2 mm before the esclerocorneal limbus parallel to the iris, taking care to not touch it or the corneal endothelium.

2.2 Vitreous Chamber Infusion

Forty-nine Perkins measurements were taken from five porcine eyes that were cannulated in the vitreous chamber at 3.5 mm from the sclerocorneal limbus with a 20-G cannula normally used in standard vitrectomy surgery.

2.3 Anatomical Study

The corneal radius and corneal thickness were measured in five porcine eyes by 11 manual keratometry (OM-4 Topcon, Japan) and ultrasound pachymetry (Sonogage Inc., Cleveland, Ohio; calibrated by the manufacturer).

2.4 Experimental Design

The eyeball was connected to a low-pressure transducer (CPC 2000, WIKA Alexander Wiegand GmbH & Co, Klingenberg, Germany) to monitor and modify the eyeball pressure. This device emits air to a conduit that communicates with a glass bottle of Ringer's lactate solution. The pressure in the glass bottle flushes the liquid by another conduit that reaches to the eyeball. The height of the serum level should be the same as the point of cannulation of the eyeball. The pressure is scheduled in the low-pressure transducer, and the whole circuit has the same pressure if differences of heights are avoided. The liquid remained free of air and vice versa as shown in Fig. 1.

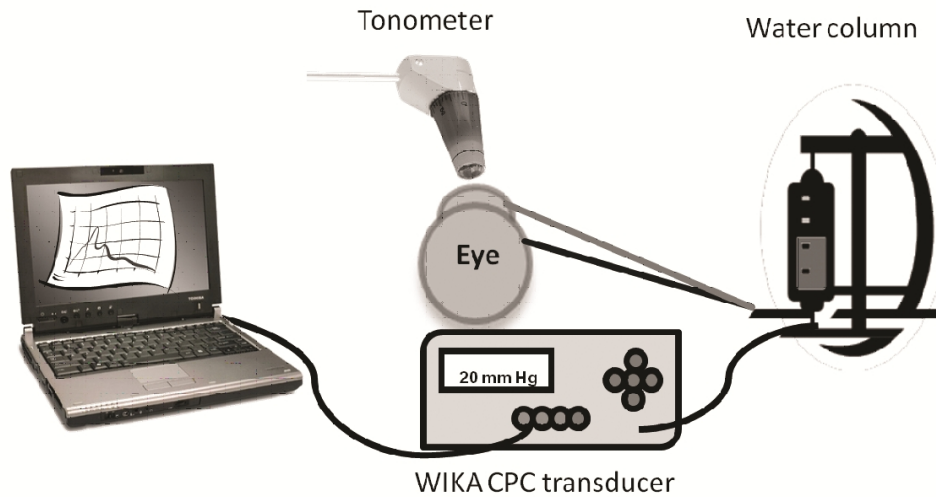


Fig. 1. Schematic representation of the experimental design

2.5 IOP Measurement

To measure IOP, a Perkins tonometer (Clement Clarke International, Edinburgh, England) calibrated by the manufacturer was used. The measurement was performed by instilling a drop of fluorescein (sodium fluorescein strips, Bausch & Lomb, dissolved in 0.9% saline solution) as a standard procedure in an eye examination.

2.6 Statistical Analysis

Statistical analysis was performed using the SPSS 15.0 (SPSS Chicago, Illinois, USA) statistical package for Windows.

The normality of the data was tested by Kolmogorov-Smirnov test. Multivariate analysis of variance (ANOVA with a Bonferroni correction for multiple comparisons) was used to detect differences between IOP measurements. The ratio of the pressure induced by the low-pressure transducer to the Perkins value was determined to detect differences between cannulations performed in the anterior chamber and in the vitreous chamber. A *p* value of less than 0.05 was considered statistically significant.

The IOP induced with the transducer (WIKA CPC 2000), when the eye was cannulated at the anterior or posterior chamber, was compared with Perkins tonometer measurements. Linear regression was used to quantify the correlation between both measurements, which was represented by the correlation coefficient (R^2). A *P* value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Working Range for the Measurement of IOP

The corneal pachymetry was $877.60 \pm 13.58 \mu\text{m}$, (95% CI 865.70 to 889.50 μm), and the corneal radii were $8.69 \pm 0.56 \text{ mm}$ (95% CI 8.94 to 8.48 mm) in the flatter meridian and $8.19 \pm 0.35 \text{ mm}$ (95% CI 8.33 to 8.06 mm) in the steeper meridian. Because the corneal thickness of the pig eye is different from the human eye, we defined a working range of pressure from 20 to 70 mm Hg with the WIKA low-pressure transducer. However, Perkins measurements showed lower pressure outcomes, ranging from 5 to 45 mm Hg.

3.2 Differences between Anterior Chamber Versus Vitreous Chamber Cannulation

No significant difference ($P=0.138$) was found between the Perkins' pressure measurements when the cannulation was in the anterior chamber and when it was in the vitreous chamber.

When the cannulation was in the anterior chamber, the pressure induced by the transducer was linearly related to the Perkins measurement ($R^2=0.940$, $P<0.001$). An equation that relates the transducer pressure to Perkins measurement was obtained: Perkins IOP= $-7.749 + 0.763$ WIKA transducer pressure as shown in Fig. 2.

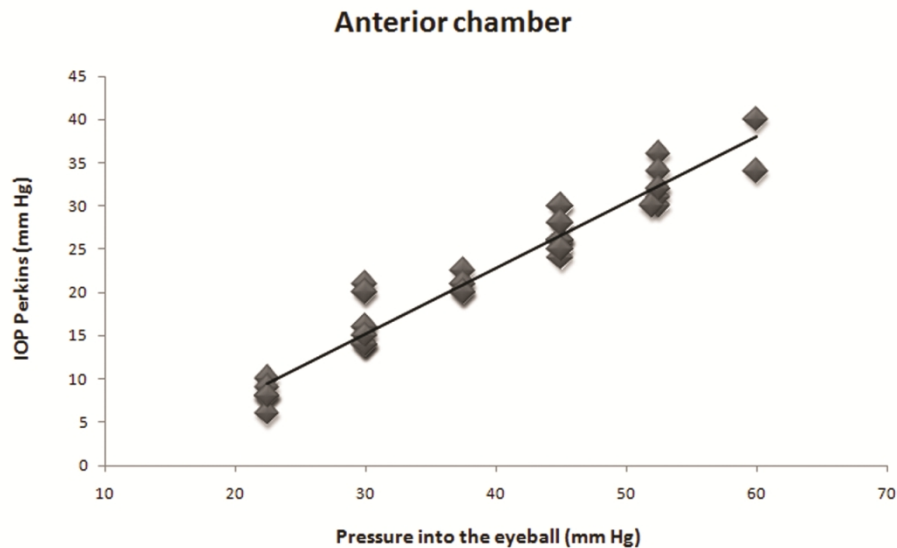


Fig. 2. Graphical representation of the equation relating the pressure generated inside the eye (x-axis) through the infusion of fluid and IOP (y-axis), taken by Perkins tonometry in eyes cannulated in the anterior chamber

When the cannulation was in the vitreous chamber, the pressure induced by the transducer was linearly related to the Perkins measurement ($R^2=0.885$, $P<0.001$). An equation that relates the transducer pressure to Perkins measurement was obtained: Perkins IOP= $-7.476 + 0.730$ WIKA transducer pressure as shown in Fig. 3.

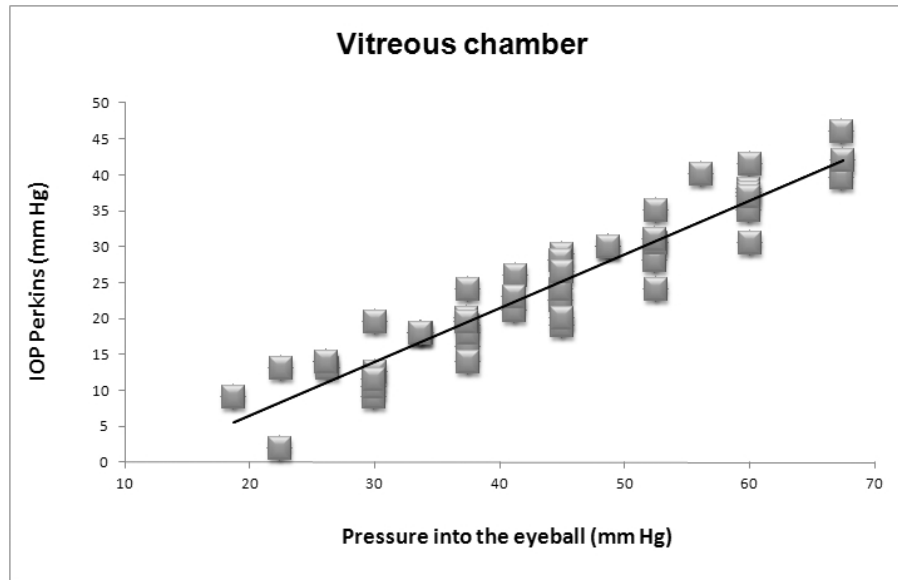


Fig. 3. Graphical representation of the equation relating the pressure generated inside the eye (x-axis) through the infusion of fluid and IOP (y-axis), taken by Perkins tonometry in eyes cannulated in the vitreous chamber

No difference was found between the ratios of Perkins to WIKA pressure ($P=0.500$) from cannulations in the anterior chamber and in the vitreous chamber.

3.3 Effect of the Eye Globe on IOP Measurement

A multiple comparison ANOVA with Bonferroni correction was conducted to test the effect of the eye globe on the IOP measurement. A significance level close to 1 ($P>0.939$) was found when comparing Perkins measurements or the Perkins/transducer ratios between the IOP measurements from cannulations in the anterior and vitreous chambers. Therefore, there were no differences in the measurements among the different eyes (pairwise comparison).

The pressure induced by the transducer was linearly related to the measurement of Perkins IOP. An equation was obtained for each eye that related the two pressures through a linear regression model with R^2 close to 1 ($P<0.001$) as shown in Table 1.

Table 1. Equation relating the pressure inside the eyeball with the Perkins IOP measurements for each measurement. Eyes 1 through 5 were cannulated in the vitreous chamber and eyes 6 through 11 in anterior chamber. P = Perkins pressure; T = transducer pressure

Eye	Equation	R ²
Vitreous chamber		
# 1	$P = -4.607 + 0.689 T$	0.954 ($P < 0.01$)
# 2	$P = -5.497 + 0.762 T$	0.949 ($P < 0.01$)
# 3	$P = -12.568 + 0.760 T$	0.938 ($P < 0.01$)
# 4	$P = -14.701 + 0.899 T$	0.960 ($P < 0.01$)
# 5	$P = -15.151 + 0.863 T$	0.966 ($P < 0.01$)
# 6	$P = -5.545 + 0.678 T$	0.993 ($P < 0.01$)
Anterior chamber		
# 7	$P = -10.750 + 0.842 T$	0.963 ($P < 0.01$)
# 8	$P = -4.845 + 0.744 T$	0.897 ($P < 0.01$)
# 9	$P = -7.076 + 0.731 T$	0.897 ($P < 0.01$)
# 10	$P = -9.286 + 0.800 T$	0.988 ($P < 0.01$)
# 11	$P = -9.714 + 0.800 T$	0.988 ($P < 0.01$)

4. DISCUSSION

The pig eye is a common model of ocular hypertension [9-13,18]. However, there is no consensus on the best way to induce IOP. Many different techniques have been reported [9-13,20], but no studies have evaluated the possible confounding effects of the method used to induce the pressure. This study helps clarify this issue and proposes a method to induce IOP that is not affected by the area of the eye in which the infusion takes place.

To calculate the range of work, a high-pressure gauge is needed to start measuring IOP with Perkins tonometry in the normal range. This is because the enucleated eye is in the phthisis, which is characterized by a lack of production of aqueous humor (the emptying of uveal vessels and choroidal flow), which is of great importance to maintain the basal pressure of the eyeball. For these reasons, it was necessary to increase the pressure inside the eyeball to recover its natural tone.

We found a direct relationship between the insufflated pressure inside the eyeball and the measurement from the outside, which could be described mathematically by the equation of a line, regardless of the point of cannulation. This result indicates that the pig eyeball meets Hooke's law for elastic bodies, in accord with previously findings [26].

No statistically significant differences were found in the pressure measured by Perkins applanation tonometry between the two areas in which the cannulation for the infusion of fluid in the pig ex vivo model eye was performed. Leaking of fluid can lead to erroneous measurements [26]. To avoid this problem, the best option could be to use a pressure system that maintains the pressure within the eyeball even if fluid is lost. Classical studies recommended gluing the cannulation point with cyanoacrylate or using some kind of dye in the liquid so that leaks can be easily perceived [26]. Also, a high-density liquid such as silicone oil has been used to obstruct the drainage pathway of aqueous humor [10,26]. In this study, the use of a low-pressure transducer ensured that the pressure within the eyeball

was kept constant in the range defined, without being influenced by leakage of intraocular fluid.

It is important to reliably know the relationship of the gauge pressure inside the eyeball with the IOP [13], as in many experimental studies with animal models that used tonometry IOP control with Tonopen [11]. This tonometer is easy to use for non-specialists in the technique of tonometry, but comparative studies with applanation tonometry values show that IOP can be underestimated, especially at pressures higher than 21 mm Hg [24,25].

Another study limitation was due to the design of the tonometers. IOP measurement with the Perkins tonometer is based in the Imbert-Fick law, which sets the IOP is the ratio between the tonometer weight and flattened area, assuming that the eye behaves like an infinitely thin, dry, elastic and spherical membrane. The cone-prism has a diameter of 7 mm, and in the process of measuring it, the corneal area is flattened and has a diameter of 3.06 mm, corresponding to the point of equality between the pressure exerted with the tonometer and IOP [27]. The Perkins tonometer is calibrated for a corneal thickness of 550 μm and corneal radius of 7.80 mm, but keratometry of the porcine cornea is flatter than human cornea, as the former has an average radius of 8.45 mm and is thicker than human cornea. For this reason, the tonometer flattened a larger diameter of 3.06 mm using a greater force with the calibrated tonometry (corneal thickness of 550 μm), which caused the difference between it and the Perkins measurements of IOP. This error will be similar for all measurements because all eyes have similar corneal thickness and radius. For this reason, this lack of Perkins measurement probably has a limited repercussion on this study's conclusions because the objective of this study was to determine whether Perkins measurement depends of the method of inducing the IOP (anterior versus vitreous chamber infusion). Also, we found the equivalence between the pressure inside the eyeball and the IOP measured by Perkins applanation tonometry that could be useful to ex vivo models, but more research is necessary to validate this equivalence in an in vivo pig model of hypertension.

5. CONCLUSION

The pressure measured by Perkins applanation tonometry in an ex vivo porcine eye model is not correlated with the region of cannulation (either anterior or vitreous chamber), keeping constant pressure with a low-pressure transducer. An equation was determined that correlates the pressure gauge with Perkins IOP, which would enable its use in studies that use the pig eye as an ex vivo animal model of hypertension in glaucoma, facilitating the interpretation of manometric measurements.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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