

Research Paper

Title: Measurement of intraocular pressure in a porcine ex vivo model eye.

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 ($IOP=-7.749+0.763\text{transducer}; R^2=0.940, p<0.001$) and when it was cannulated in the vitreous chamber ($IOP=-7.476+0.730\text{transducer}, R^2=0.885, p<0.001$). 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 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 exvivo animal model of hypertension in glaucoma.

Keywords: ex vivo animal model, glaucoma model, hypertension model, pig eye animal model.

1. INTRODUCTION

Glaucoma is a 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. MATERIAL AND METHODS

76 The study was conducted in IOBA Eye Institute, University of Valladolid (Spain).

77 Eleven enucleated pig eyes (*Sus scrofa domestica*) were obtained from the
78 local abattoir. Animals were white (not albino) domestic pigs between six and
79 eight months of age, and they weighed 120 to 150 kg. The eyes were
80 enucleated around 8:30 AM, just after slaughter. The measurements were
81 made between 9:00 AM and 12:00 AM. Excess tissue surrounding the eyeball
82 was removed with Moorfield conjunctival forceps and Wescott tenotomy wide-
83 handle scissors (John Weiss international, Milton Keynes, UK) to facilitate the
84 experimental measurements.

85

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

90

91 2.1. Anterior chamber infusion

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

96

97 2.2. Vitreous chamber infusion

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

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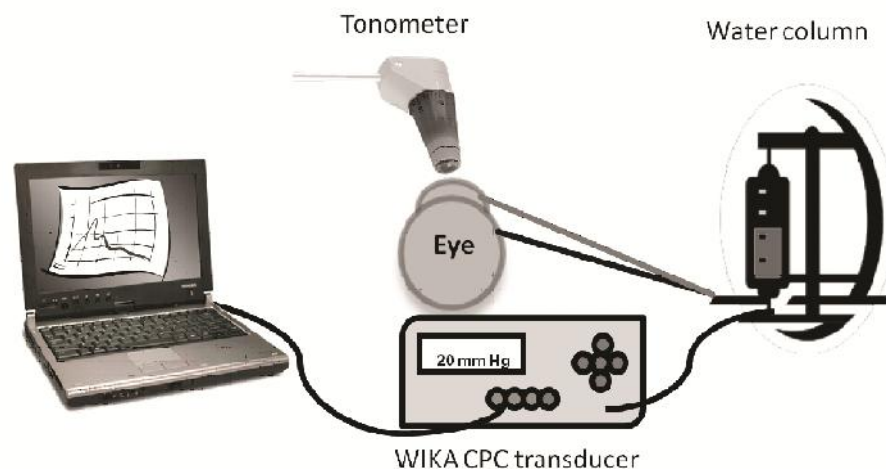
104 2.3. Anatomical study

105 The corneal radius and corneal thickness were measured in five porcine eyes
106 by11 manual keratometry (OM-4 Topcon, Japan) and ultrasound pachymetry
107 (Sonogage Inc., Cleveland, Ohio; calibrated by the manufacturer).

108

109 2.4. Experimental design

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



119

120 **Figure 1.** Schematic representation of the experimental design.

121

122

123 2.5. IOP measurement

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

129

130 2.6. Statistical analysis

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

133

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

141

142 The IOP induced with the transducer (WIKA CPC 2000), when the eye was
143 cannulated at the anterior or posterior chamber, was compared with Perkins
144 tonometer measurements. Linear regression was used to quantify the

145 correlation between both measurements, which was represented by the
146 correlation coefficient (R^2). A p value of less than 0.05 was considered
147 statistically significant.

148 **3. RESULTS**

149

150 3.1. Working range for the measurement of IOP

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

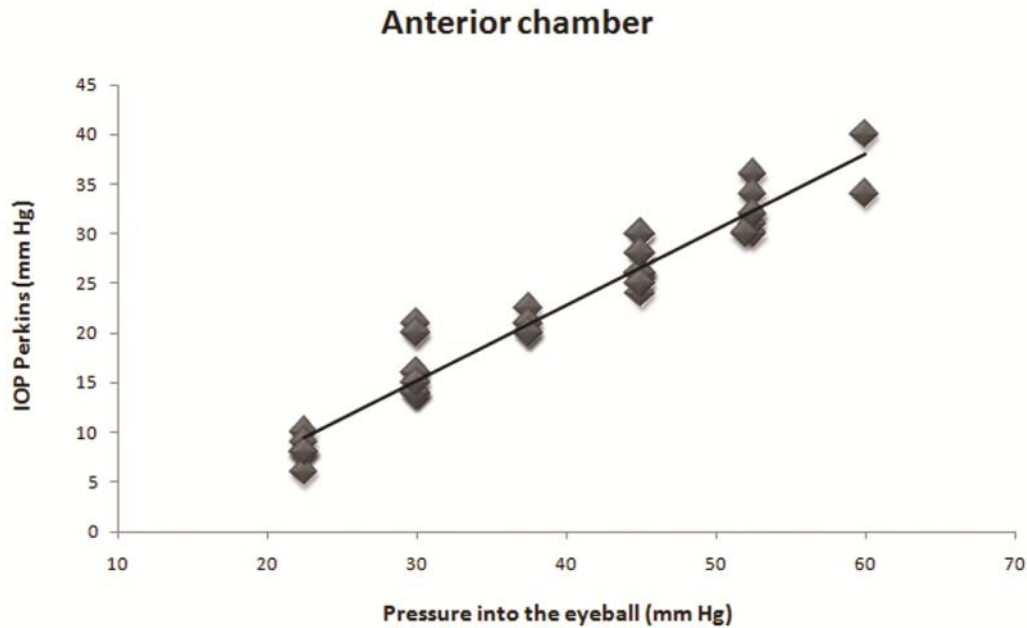
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159 3.2. Differences between anterior chamber versus vitreous chamber 160 cannulation

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

164

165 When the cannulation was in the anterior chamber, the pressure induced by the
166 transducer was linearly related to the Perkins measurement ($R^2=0.940$,
167 $p<0.001$). An equation that relates the transducer pressure to Perkins
168 measurement was obtained (Figure 2): Perkins IOP= $-7.749 + 0.763$ WIKA
169 transducer pressure.

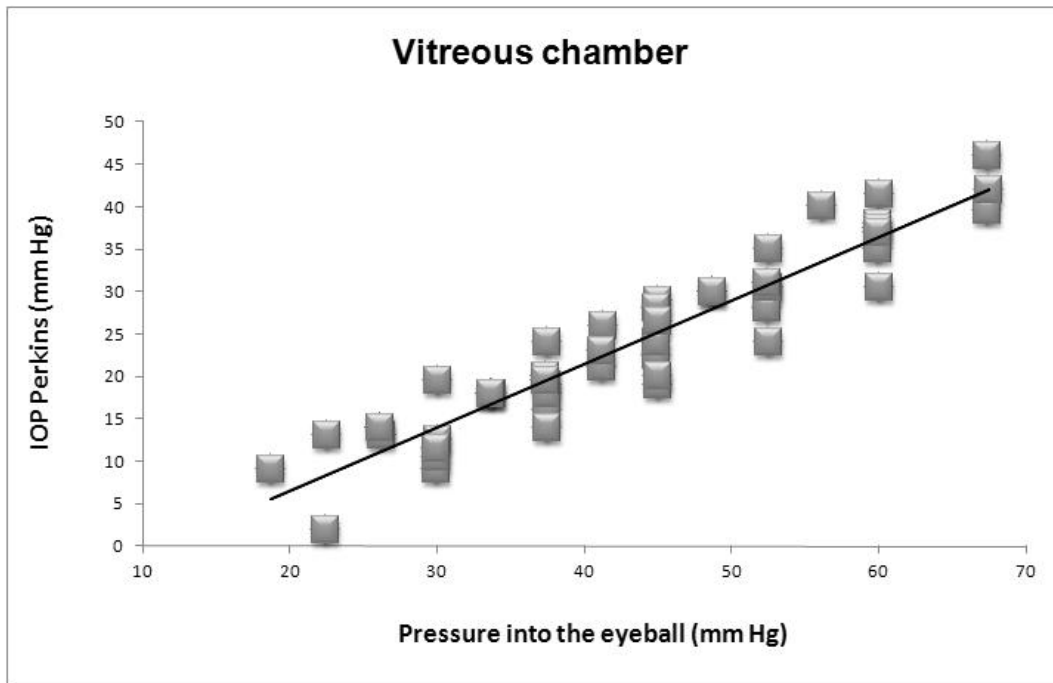


170

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

174

175 When the cannulation was in the vitreous chamber, the pressure induced by the
 176 transducer was linearly related to the Perkins measurement ($R^2=0.885$,
 177 $p<0.001$). An equation that relates the transducer pressure to Perkins
 178 measurement was obtained (Figure 3): Perkins IOP= $-7.476 + 0.730$ WIKA
 179 transducer pressure.



180

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

185

186 No difference was found between the ratios of Perkins to WKA pressure
 187 ($p=0.500$) from cannulations in the anterior chamber and in the vitreous
 188 chamber.

189

190 3.3. Effect of the eye globe on IOP measurement

191 A multiple comparison ANOVA with Bonferroni correction was conducted to test
 192 the effect of the eye globe on the IOP measurement. A significance level close
 193 to 1 ($p>0.939$) was found when comparing Perkins measurements or the
 194 Perkins/transducer ratios between the IOP measurements from cannulations in

195 the anterior and vitreous chambers. Therefore, there were no differences in the
 196 measurements among the different eyes (pairwise comparison).

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

201

<u>Eye</u>	<u>Equation</u>	<u>R²</u>
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 ($p < 0.01$)

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

206

207 **4. DISCUSSION**

208

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

215

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

223

224 We found a direct relationship between the insufflated pressure inside the
225 eyeball and the measurement from the outside, which could be described
226 mathematically by the equation of a line, regardless of the point of cannulation.

227 This result indicates that the pig eyeball meets Hooke's law for elastic bodies, in
228 accord with previously findings [26].

229 No statistically significant differences were found in the pressure measured by
230 Perkins applanation tonometry between the two areas in which the cannulation
231 for the infusion of fluid in the pig ex vivo model eye was performed. Leaking of
232 fluid can lead to erroneous measurements [26]. To avoid this problem, the best
233 option could be to use a pressure system that maintains the pressure within the
234 eyeball even if fluid is lost. Classical studies recommended gluing the
235 cannulation point with cyanoacrylate or using some kind of dye in the liquid so
236 that leaks can be easily perceived [26]. Also, a high-density liquid such as
237 silicone oil has been used to obstruct the drainage pathway of aqueous humor
238 [10,26]. In this study, the use of a low-pressure transducer ensured that the
239 pressure within the eyeball was kept constant in the range defined, without
240 being influenced by leakage of intraocular fluid.

241

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

248

249 Another study limitation was due to the design of the tonometers. IOP
250 measurement with the Perkins tonometer is based in the Imbert-Fick law, which
251 sets the IOP is the ratio between the tonometer weight and flattened area,

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

272

273 In conclusion, the pressure measured by Perkins applanation tonometry in an
274 ex vivo porcine eye model is not correlated with the region of cannulation (either
275 anterior or vitreous chamber), keeping constant pressure with a low-pressure
276 transducer. An equation was determined that correlates the pressure gauge

277 with Perkins IOP, which would enable its use in studies that use the pig eye as
278 an ex vivo animal model of hypertension in glaucoma, facilitating the
279 interpretation of manometric measurements.

280

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