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# The pig eye as a novel model of glaucoma

Javier Ruiz-Ederra[a], Mónica García[a], María Hernández[a], Haritz Urcola[a], Ernesto Hernández-Barbáchano[a], Javier Araiz[b], Elena Vecino[a,*]

[a]Department of Cellular Biology, Faculty of Medicine, University of the Basque Country, E-48940 Leioa, Vizcaya, Spain
[b]Department of Ophthalmology, Faculty of Medicine, University of the Basque Country, E-48940 Leioa, Vizcaya, Spain

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**Abstract**

We validated the pig eye as a model of glaucoma, based on chronic elevation of intraocular pressure (IOP). IOP was elevated by cauterising three episcleral veins in each of the left eyes of five adult pigs. Right eyes were used as controls. Measurement of IOP was performed during the experiment with an applanation tonometer (Tono-Pen). Five months after episcleral vein occlusion, retinal ganglion cells (RGCs) from both cauterised and control eyes were retrogradely backfilled with Fluoro-Gold. Analysis of RGC loss and morphometric as characterization of surviving RGCs was performed using whole-mounted retinas. Elevation of IOP was apparent after three weeks of episcleral vein cauterisation and it remained elevated for at least 21 weeks (duration of the experiments). Analysis of RGC loss after chronic elevation of IOP revealed that RGC death was significant in the mid-peripheral and peripheral retina, mainly in the temporal quadrants of both retinal regions. Moreover the mean soma area of remaining RGCs was observed to increase and we found a greater loss of large RGCs in the mid-peripheral and peripheral retina. We conclude that the pattern of RGC death induced in the pig retina by episcleral vein cauterisation resembles that found in human glaucoma. On the basis of this study, the pig retina may be considered as a suitable model for glaucoma-related studies, based on its similarity with human and on its affordability.


*Keywords:* fluoro-gold; intraocular pressure; porcine; tracer; RGC; glaucoma

## 1. Introduction

Glaucoma is the second most common cause of blindness worldwide, after cataract (Weinreb and Khaw, 2004). This ocular disease is associated with a progressive loss of the visual field, caused by retinal ganglion cell (RGC) death. Increased intraocular pressure (IOP) constitutes one of the principal risk factors (Glovinsky et al., 1991; Vickers et al., 1995; Wygnanski et al., 1995). Consequently, most of the current therapies to treat glaucoma are directed to lowering IOP, in order to minimise cell death. Thus, useful models of glaucoma inevitably involve a significant and sustained elevation of IOP.

The only large mammal, which is currently being employed for the induction of experimental glaucoma, is the monkey (Kalvin et al., 1966; Glovinsky et al., 1991; Morgan et al., 2000; Kashiwagi et al., 2003). Despite being an excellent model, monkey availability is very low due to ethical and economical reasons. Thus, it is of interest to evaluate the suitability of the pig as a model of glaucoma, since it is phylogenetically close to the human and is much more available than the monkey. The pig eye/retina shares many similarities with that of the human (Prince et al., 1960; Beauchemin, 1974; Peichl et al., 1987; De Schaepdrijver et al., 1990; McMenamin and Steptoe, 1991; Olsen et al., 2002; Ruiz-Ederra et al., 2003, 2004; Garcia et al., 2005). The porcine retina is even more similar to the human retina than that of other large mammals such as the dog, goat, cow or ox (Prince et al., 1960). Moreover the pig has recently been used to genetically reproduce a retinitis pigmentosa condition, similar to that found in human (Li et al., 1998). Additionally, tools employed for diagnostics in ophthalmology, such as optical coherence tomography, corneal topography imaging or multi-focal electroretinography can

* Corresponding author. Address: Department of Cell Biology and Histology, Faculty of Medicine, University of the Basque Country, E-48940 Leioa, Vizcaya, Spain
*E-mail address:* gcpvecoe@lg.ehu.es (E. Vecino).

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be applied to the pig eye, supporting the use of this animal as a good model for ophthalmological studies (Kyhn et al., IOVS, 2004, 2, 'ARVO E-abstract', 4247; Maverick et al., IOVS, 2004, 2, 'ARVO E-abstract', 2876; Van Velthoven et al., IOVS, 2004, 2, 'ARVO E-abstract', 2371). Finally, studies of the pig aqueous outflow system showed that this animal could be a suitable model for specific types of glaucoma (McMenamin and Steptoe, 1991).

In a previous study, we reported the presence of three classes of RGCs based on soma size (small, medium and large) (Garcia et al., 2002) and performed a detailed study of the pig RGC topography as a function of soma size. Our study revealed that the distribution of the different sized RGCs is very similar in the porcine and human retina (Garcia et al., 2005). This information may be useful in order to unravel the mechanisms implicated in the selective death of some size groups of RGCs in glaucoma, since it is generally accepted that large RGCs are more susceptible to death during human or experimental glaucoma (Quigley et al., 1987, 1988, 1989; Glovinsky et al., 1991; Vickers et al., 1995).

In the present work, we have evaluated the pig eye as a novel model of glaucoma. Our study indicates that the pig eye is a suitable animal model for glaucoma experimentation, based on the similarity of the features observed in human glaucoma and in the pig eye subjected to chronic increased intraocular pressure, and on the more ready availability of pig eyes in comparison to those of non-human primates.

## 2. Materials and methods

All experiments were conducted following the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. We induced a chronic elevation IOP within pig eyes by means of episcleral vein occlusion. Measurements of IOP as well as analysis of optic disc excavation were performed through the experimental period. At the end of this period, RGC death was measured by analysing RGCs, which had been retrogradely back-filled with Fluoro-Gold. The eyecups were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer saline (PBS, pH 7.4) for 4 hr at 4°C and then retinas were removed and flat-mounted with the retinal ganglion cell layer being uppermost. They were then cover slipped with PBS/glycerine (1:1).

### 2.1. Induction of experimental glaucoma

Five adult pigs (*Sus scrofa*) were used in the present work. An increase in IOP was induced by cauterising three episcleral veins of the left eyes of the animals following the method described elsewhere (Shareef et al., 1995). Briefly, pigs were deeply anaesthetised following the protocol described above, with an intramuscular injection of ketamine hydrochloride (Ketolar) + xylazine (Diazepan) (each 20 mg $kg^{-1}$). An intravenous cannula was applied to the ear in order to provide the animal with additional anaesthetic (1 ml Propofol every 15 min), maintaining deep anaesthesia throughout the operation. A life-support machine was used to facilitate breathing and to monitor vital functions during the operation. Three episcleral veins (nasal, dorsal and temporal) were cauterised following the protocol described by Shareef et al. (1995) in rats. Animals were kept alive during 21 weeks after episcleral vein occlusion.

### 2.2. Intraocular pressure measurement

IOP was measured with an applanation tonometer (Tono-Pen XL; Medtronic, Jacksonville, FL, USA) under light general anaesthesia (Ketamine + Xilazine), following application of drops of tetracaine hydrochloride (1 mg $ml^{-1}$) + oxibuprocaine hydrochloride (4 mg $ml^{-1}$) on the corneas (Colircusí, Alcon Cusí, Spain). All measurements were carried out at the same time and always before feeding the animals. Forty-five days before the episcleral vein operation, the IOP of both eyes was measured in order to obtain the baseline values. One week post-operation, both right (non-operated control) and left (cauterised) eyes were measured at fortnight intervals, to evaluate the increase in IOP. The tonometer was applied perpendicularly to the more apical side of the cornea, until at least five or six independent measurements were obtained (each of these IOP values was the average of four IOP readings. The results of the IOP reading were accepted if the confidence interval was greater than or equal to 95%). The mean values of the IOP measurements were eventually averaged, and results were expressed as mean IOP ± SEM. Five such measurements were made.

### 2.3. Capture of eye fundus images

In order to follow-up the progression of the excavation of the papilla, we captured images from the optic disc of control and cauterised eyes, 1 week after the occlusion of the episcleral veins and 1 week before the end of the IOP increase period. Images were obtained under general anaesthesia, using a hand held fundus camera. Optic disc excavation was determined comparing cup/disc ratio values at initial stages and at final stages of the glaucomatous procedure. Measurements were performed using a digital palette (Easypen, Genius) in combination with image analysis software (Scion Image; Scion, Frederick, MD) for digitised images.

### 2.4. RGC backfilling

RGCs from eight eyes were backfilled from the optic nerve with 3% Fluoro-Gold (Fluorochrome, Englewood CO, USA) diluted in a solution containing 0.9% NaCl and 0.1% dimethylsulfoxide. Forty microlitres of Fluoro-Gold