The pig eye as a novel model of glaucoma

Javier Ruiz-Ederra\textsuperscript{a}, Mónica García\textsuperscript{a}, María Hernández\textsuperscript{a}, Haritz Urcola\textsuperscript{a}, Ernesto Hernández-Barbáchano\textsuperscript{a}, Javier Araiz\textsuperscript{b}, Elena Vecino\textsuperscript{a,*}

\textsuperscript{a}Department of Cellular Biology, Faculty of Medicine, University of the Basque Country, E-48940 Leioa, Vizcaya, Spain
\textsuperscript{b}Department of Ophthalmology, Faculty of Medicine, University of the Basque Country, E-48940 Leioa, Vizcaya, Spain

Received 17 November 2004; accepted in revised form 29 March 2005
Available online 9 June 2005

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 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.

\$2005$ Elsevier Ltd. All rights reserved.

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 Schaedtli and Steptoe, 1991; 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...
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 optic disc excavation and measured through the experimental period. At the end of this period, RGC death was measured by optic disc excavation and mean IOP were measured perpendicularly 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.

2.1. 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...
was injected into the optic nerve around 4 mm from the optic nerve head. Pigs were kept alive for two days post-operation to allow Fluoro-Gold to fill the entire population of RGCs. Then animals were euthanised with an overdose of anaesthesia, the eyes were enucleated and the lens and vitreous were extracted by cutting the anterior chamber at the level of the ora serrata. 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), so that shrinkage did not occur during the processing of the tissue.

We divided the animals in two groups: the first one consisted of two pigs, whose RGCs from their left (cauterised) eyes were backfilled with Fluoro-Gold. RGC topography pertaining to cauterise eyes was then compared with that of a pool of control eyes from different pigs, which we had analysed in previous studies. The second group consisted of three pigs, whose RGCs from both left and right eyes were backfilled from the optic nerve with the fluorescent tracer; a paired analysis of RGC distribution between fellow eyes was then performed. Since we only found differences in the number and distribution of RGCs when we compared fellow control vs. cauterised eyes, the results in the present study regarding RGC topography will refer to the paired study performed in this group.

2.5. Capture of retinal ganglion cell images

Images from both the left and the right retinas of each animal were obtained using an epifluorescence microscope (Axioskop 2; Zeiss, Jena, Germany) coupled to a digital camera (Coolsnap, RS Photometrics, Tucson, USA). The images were captured in a systematic way using the optic disc and the visual streak as reference points, as previously described (Garcia et al., 2005). Briefly, we recorded one out of four 40× microscope fields from the optic disc towards the periphery, along both the X and the Y-axes of the retina. We recorded 100–130 fields/retina, using the 40× objective. The sampling area recorded represented 1.4% of the mean area of the six retinas analysed. This sample size has been previously reported to represent a significant percentage of the retina (Peinado-Ramón et al., 1996).

2.6. Morphometric analysis

Morphometric analysis was performed as described elsewhere (Garcia et al., 2002). For each recorded retinal field, we quantified the number of RGCs and the soma area of each of the RGCs present. Analysis of these two parameters was performed using a digital palette (Easypen, Genius, Taipei, Taiwan) in combination with image analysis software (Scion Image; Scion, Frederick, MD). Each RGC soma was filled out directly on the computer screen, so that area and major axis length could be measured. Values for both parameters were transferred to a data sheet for subsequent statistical analysis.

RGC density, mean area and soma size distribution (attending to major axis length in small, medium and large RGCs: <15; 15–20 and >20 μm, respectively) were analysed separately in the three major regions of the retina: the visual streak, mid-periphery and periphery (Unpublished results). We proceeded with further subdivision in quadrants (nasal–dorsal (ND); nasal–ventral (NV); temporal–dorsal (TD) and temporal–ventral (TV) only in those retinal regions where we found significant differences with respect to controls.

2.7. Statistical analysis

RGC density, mean soma area and percentage of size groups, as well as IOP measurements were compared between glaucomatous and control eyes, by a paired Student test, using SPSS software (SPSS Sciences, Chicago, IL). Values are expressed as mean±SEM. The minimum level of significant difference was defined as p<0.05.

3. Results

3.1. Intraocular pressure

The mean IOP in control eyes was 15.2±1.8 mmHg. Elevation of IOP after cauterisation of three episcleral veins was apparent by the third week in all animals, when IOP rose from 15.6±1.8 mmHg in control eyes to 20.8±2.4 mmHg in cauterised eyes (1.3 fold-increase, p=0.032). Differences reached a maximum by the 16th week with a 1.4 fold-increase, p=0.048 (21.0±4.1 mmHg (cauterised) vs. 14.8±1.3 mmHg (control)). Elevation of IOP was maintained throughout the course of the experiment, with significant differences still being apparent at week 20 (15.1±2.9 mmHg in control vs. 19.8±5.9 mmHg in cauterised eyes, p=0.037) and week 21 (11.5±0.9 mmHg in control vs. 13.9±0.8 mmHg in cauterised eyes, p=0.021) (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Week</th>
<th>Mean IOP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Cauterised IOP fold-increase</td>
</tr>
<tr>
<td>Baseline</td>
<td>15.2±2.1</td>
</tr>
<tr>
<td>3</td>
<td>15.6±1.7</td>
</tr>
<tr>
<td>9</td>
<td>15.0±1.7</td>
</tr>
<tr>
<td>12</td>
<td>15.5±3.2</td>
</tr>
<tr>
<td>16</td>
<td>14.8±1.5</td>
</tr>
<tr>
<td>20</td>
<td>15.1±2.3</td>
</tr>
<tr>
<td>21</td>
<td>11.5±0.7</td>
</tr>
</tbody>
</table>

Values are expressed as mean IOP (mmHg)±SEM. Results from statistical analysis are represented by *p<0.05, significant difference with respect to control.
3.2. Eye fundus

Examination of the eye fundus showed a discrete excavation of the optic disc of all glaucomatous eyes at advanced stages of optic nerve damage \((p = 0.055)\). Thus, the cup/disc ratio varied from 0.58 ± 0.04 one week after the onset of IOP elevation to 0.63 ± 0.04 at week 21 following vein cauterisation. Optic disc excavation was apparent from the curving of the blood vessels (especially the thinner ones) at the disc margin in the glaucomatous eyes at advanced stages of damage (Fig. 1). However, blood supply did not seem to be affected since we could identify all the large and small blood vessels after the period of IOP elevation, which were indistinguishable from those of the control retinas (Fig. 1).

3.3. Pattern of retinal ganglion cell death

Chronic elevation of IOP led to a mean loss of 18 ± 8%, \(p = 0.0005\) of RGCs in the glaucomatous retinas. RGC loss did not take place homogeneously, but was more pronounced in the peripheral and mid-peripheral retina, compared to the more central retina where the visual streak (a high RGC density region) is located. Moreover, a larger loss of RGCs occurred in the temporal retina, which was associated with an increase in the mean soma area of RGCs. The combination of both phenomena resulted in an alteration in the proportion of RGC size groups along the retina.

3.4. Retinal ganglion cell density loss

RGC loss in glaucomatous compared to control retinas was significantly greater in peripheral regions. Thus, RGC density in the control peripheral retina was 239 ± 28 RGCs per mm\(^2\), whereas in cauterised eyes, RGC density was significantly reduced (188 ± 23 RGCs per mm\(^2\)) (21% loss, \(p = 0.046\)). This situation was also observed in the mid-periphery (727 ± 27 vs. 838 ± 34 RGCs per mm\(^2\) in control eyes; a 13% loss, \(p = 0.0005\)). No significant decrease in RGC density was observed within the visual streak (4216 ± 148 vs. 4285 ± 139 RGCs per mm\(^2\) in control eyes) (Fig. 2A).
A more detailed analysis of the different regions of the mid-peripheral retina revealed that RGC loss was greater in both temporal quadrants with significant cell loss in the temporal–dorsal (TD) quadrant (1015 ± 62 vs. 1310 ± 67 RGCs per mm² in control eyes; 22.5% decrease, \( p = 0.0005 \)), and in the temporal–ventral (TV) quadrant (428 ± 40 vs. 645 ± 78 RGCs per mm² in control eyes; 33.6% loss, \( p = 0.01 \)) (Figs. 2B and 3A). No differences were observed in the quadrants located in the nasal retina.

Although differences in RGC density in the peripheral retina were found to be significant (Fig. 2A), no significant differences were found when we analysed each retinal quadrant separately, probably due to the small number of RGCs located in the fields captured in the periphery. However, the density of RGCs was found to be reduced in the TD and TV quadrants of glaucomatous retinas compared to controls. Thus, we observed an RGC density of 270 ± 70 vs. 377 ± 87 RGCs per mm² in control eyes in TD quadrant (28.3% decrease, \( p = 0.30 \)), and 154 ± 73 vs. 282 ± 77 RGCs per mm² in controls eyes (45% decrease, \( p = 0.45 \)) in the TV quadrant (Figs. 2C and 3B).

Fig. 3. Representative images from the mid-peripheral (A) and peripheral (B) retina corresponding to both temporal quadrants in control (left) and cauterised (right) porcine eyes captured at an equivalent retinal location. Porcine retinal ganglion cells from both control and cauterised eyes were retrogradely backfilled with Fluoro-Gold. Abbreviations: TD, temporal–dorsal; TV, temporal–ventral. Scale bar in B represents 20 μm for all images.
3.5. Changes in retinal ganglion cell mean area

The mean area of RGC somata increased in the experimental retinas with respect to controls. This increase was observed in the mid-periphery where we detected a mean RGC soma area of \(235 \pm 3 \mu m^2\) vs. \(244 \pm 4 \mu m^2\) in controls (a 3.8% increase, \(p=0.01\)) and in the periphery (\(328 \pm 16\) vs. \(298 \pm 8 \mu m^2\) in controls; a 10% increase, \(p=0.048\)). No significant differences were observed in mean RGC area in the visual streak (\(170 \pm 4\) vs. \(179 \pm 4 \mu m^2\) in controls) (Fig. 4A).

Upon analyzing the mid-peripheral regions in detail, we found a significant increase in the mean area of RGC somata in quadrants located on the temporal side of the experimental retina. Thus, in the TD quadrant, we found a mean RGC soma area of \(249 \pm 5\) vs. \(229 \pm 4 \mu m^2\) in control eyes (8.7% increase, \(p=0.004\)). In the TV quadrant, we measured a mean RGC soma area of \(289 \pm 16\) vs. \(228 \pm 10 \mu m^2\) in control eyes (27% increase, \(p=0.003\)).

Significant differences were not observed in the nasal retina (Fig. 4B).

With respect to the peripheral retina, we observed an increase of the mean area of RGC somata in quadrants located in the temporal side of the glaucomatous retina, in comparison with the corresponding controls. Thus, the mean RGC soma area was \(281 \pm 13\) (cauterised) vs. \(263 \pm 23 \mu m^2\) (control) in the TD quadrant (6.8% increase, \(p=0.48\)) and \(320 \pm 21\) (cauterised) vs. \(304 \pm 17 \mu m^2\) (control) in the TV quadrant (5.3% increase, \(p=0.31\)). However, as occurred with the analysis of RGC density in the periphery, the small number of RGCs present in fields located in the periphery lead to large variability, which did not allow us to observe significant differences. Differences were not observed in the nasal retina (Fig. 4C).

3.6. Retinal ganglion cell loss as a function of soma size

We analysed RGC loss in terms of soma size, by analyzing the density of large, medium and small RGCs as well as the percentages of these groups of RGCs in the three retinal regions and in the temporal quadrants. We observed a significant loss of large RGCs in the visual streak, with \(475 \pm 42\) large RGCs per mm\(^2\) in controls vs. \(333 \pm 42\) large RGCs per mm\(^2\) in cauterised eyes (29% loss, \(p=0.004\)) (Fig. 5). The percentage of large RGCs, which represents \(11.1 \pm 1.5\%\) of total RGCs in the control visual streak, was significantly (\(p=0.005\)) reduced in the visual streak of cauterised eyes, representing \(7.9 \pm 1.4\%\) of total RGCs in this retinal region (Table 2A).

Significant (\(p=0.007\)) loss of large RGCs was also observed in the mid-peripheral retina, with \(266 \pm 9\) large
Table 2  
Percentages of small, medium and large retinal ganglion cells within the visual streak (A), and the different quadrants of the mid-periphery (B) and periphery (C) of control and cauterised eyes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cauterized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(A) Percentage of visual streak RGCs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS Large (&gt;21 mm)</td>
<td>11.1 ± 1.5</td>
<td>7.9 ± 1.4**</td>
</tr>
<tr>
<td>Medium size (15–20 mm)</td>
<td>53.0 ± 2.0</td>
<td>53.6 ± 2.0</td>
</tr>
<tr>
<td>Small (&lt;14 mm)</td>
<td>35.9 ± 2.3</td>
<td>38.5 ± 2.2</td>
</tr>
<tr>
<td><strong>(B) Percentage of mid-periphery RGCs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD Large (&gt;21 mm)</td>
<td>32.8 ± 1.2</td>
<td>37.6 ± 1.9**</td>
</tr>
<tr>
<td>Medium size (15–20 mm)</td>
<td>40.3 ± 1.6</td>
<td>42.9 ± 1.5</td>
</tr>
<tr>
<td>Small (&lt;14 mm)</td>
<td>26.4 ± 1.8</td>
<td>19.5 ± 1.4**</td>
</tr>
<tr>
<td>TV Large (&gt;21 mm)</td>
<td>33.5 ± 2.1</td>
<td>49.1 ± 4.5**</td>
</tr>
<tr>
<td>Medium size (15–20 mm)</td>
<td>40.1 ± 3.3</td>
<td>39.1 ± 3.6</td>
</tr>
<tr>
<td>Small (&lt;14 mm)</td>
<td>26.5 ± 4.5</td>
<td>11.7 ± 2.1**</td>
</tr>
<tr>
<td><strong>(C) Percentage of peripheral RGCs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD Large (&gt;21 mm)</td>
<td>53.1 ± 9.9</td>
<td>65.9 ± 11.1</td>
</tr>
<tr>
<td>Medium size (15–20 mm)</td>
<td>39.4 ± 8.8</td>
<td>17.3 ± 7.9</td>
</tr>
<tr>
<td>Small (&lt;14 mm)</td>
<td>7.5 ± 4.2</td>
<td>16.9 ± 6.9</td>
</tr>
<tr>
<td>TV Large (&gt;21 mm)</td>
<td>54.1 ± 9.9</td>
<td>73.8 ± 11.1</td>
</tr>
<tr>
<td>Medium size (15–20 mm)</td>
<td>30.3 ± 7.7</td>
<td>16.8 ± 8.4</td>
</tr>
<tr>
<td>Small (&lt;14 mm)</td>
<td>15.6 ± 6.8</td>
<td>9.4 ± 5.5</td>
</tr>
</tbody>
</table>

Values are the mean value of the percentages of different sized RGCs ± sst. Results from statistical analysis are represented by: **p < 0.01 significant difference with respect to control retinas.

RGCs per mm² in controls vs. 242 ± 9 large RGCs per mm² in cauterised eyes. Moreover, we found a significant (p = 0.0005) loss of small RGCs in this region after cauterisation of episcleral veins, with 238 ± 50 small RGCs per mm² in controls vs. 155 ± 25 small RGCs per mm² in cauterised eyes (Fig. 5). A detailed analysis of the mid-periphery showed that the greater changes in the percentage of RGCs in terms of soma size took place in the temporal quadrants (Table 2B).

Finally, although we did not observe significant differences in the density of small, medium or large RGCs in the peripheral retina (Fig. 5), variations in the distribution of percentages of RGC soma size (Table 2) followed a similar trend to that observed in the mid-periphery after elevation of IOP. However, differences were not significant probably due to the small number of RGCs located in the fields captured in this retinal region.

4. Discussion

In the present work, we have established a model of glaucoma based on the occlusion of the episcleral vein method, using the pig as a novel experimental animal. Damage was considered to be glaucomatous due to the presence of elevated IOP, altered eye fundus morphology and RGC loss.

Elevation of IOP was observed after the third week with values 1.3 times higher in cauterised than in control eyes. By the 16th week, IOP was 1.4 times higher than in the control eye. Differences in the IOP were maintained up to the end of the experimental period, with significant differences between glaucomatous and control eyes being present at the 21st week. This IOP fold-increase is similar to that reported by others for rat eyes with experimental glaucoma induced via the same method to increase the IOP (Laquis et al., 1998). These authors described a loss of RGCs in association with elevated IOP. Previous studies of monkey eyes with experimental glaucoma showed that the IOP in hypertensive eyes was about 2.1 or 2.4 times higher than the IOP in control eyes (Pease et al., 2000; Weber et al., 1998). However, the method employed to increase the monkey IOP was different to that used in the present study. Additionally, it is quite possible that Schlemm’s canal in the pig eye, which is made up of multiple collector vessels (McMenamin and Steptoe, 1991) could at least in part, correct the increase of IOP induced by the cauterisation of the episcleral veins in the pig eye.

The glaucomatous eye fundus revealed optic disc excavation, which is one of the most common events associated with fibre loss in glaucoma. This was observed in all glaucomatous animals analysed. The mean values of the cup/disc ratio were 0.58 ± 0.04 at the initial state of glaucomatous damage and 0.63 ± 0.04 at later, more advanced stages. The increase in optic disc excavation was not very prominent, if we compare with optic disc excavation reported previously in monkey under experimental glaucoma (Weber and Zelenak, 2001). However, it has been reported that topographic features of the optic disc of patients of different races convey different information with regard to assessing the risk of early glaucoma. Therefore, it is possible that the changes in the optic disc topography parameters associated with the elevation of IOP are not indicative of the glaucomatous damage in the porcine retina (Girkin et al., 2003).

4.1. Retinal ganglion cell loss in glaucomatous eyes

Elevation of IOP led to RGC loss, which was significant in peripheral regions of the retina (the mid-periphery and the peripheral retina). A greater loss of RGCs in the peripheral retina of monkey and rat with experimental glaucoma has previously been reported (Laquis et al., 1998; Vickers et al., 1995). A more detailed analysis revealed that regions located in the temporal quadrants showed a greater loss of RGCs. This finding correlates with previous reports, in which a greater loss of RGC fibres and loss of visual function were reported for the temporal compared to the nasal human retina (Fortune et al., 2002; Garway-Heath et al., 2002; Takamoto and Schwartz, 2002).

4.2. Increase in the mean soma area of the remaining RGCs

We observed an increase in the mean area of RGC somata in those regions of the retina, which presented significant RGC loss. An increment in RGC mean soma area, associated with a decrease in RGC density, has
induced glaucoma (Asai et al., 1987; Glovinsky et al., 1993). The same phenomenon has been reported using the rat episcleral vein cauterisation glaucoma model and the authors suggested that the increment of RGC soma size intrinsically linked to soma density, was probably a compensatory response to cell loss (Ahmed et al., 2001). It is possible that the remaining RGCs are stimulated to occupy the areas remaining after RGC death, increasing their soma area and/or dendritic field, as described by other authors (Perry and Linden, 1982; Kirby and Chalupa, 1986; Ahmed et al., 2001).

Alternatively, the increase in RGC volume before death could represent a loss of osmotic regulation, secondary to RGC damage (Morgan et al., 2000). In contrast, a reduction in the mean soma size of the remaining RGCs after induction of experimental glaucoma in the monkey and in the cat has been reported (Weber et al., 1998; Shou et al., 2003). It is possible that the mean volume/area values of glaucomatous RGCs are dependent on the stage at which the disease is studied.

4.3. Selective RGC loss

Our analysis of RGC loss in terms of soma size points to a selective death of large RGCs. This was especially evident in the visual streak, in which a significant loss of large RGCs was found. The fact that the mean area of RGCs located in the visual streak did not increase after the experimental period may possibly indicate that this region of the retina is experiencing the initial stages of damage due to increased IOP, while more advanced stages of tissue damage (and increased mean RGC area) are evident in the peripheral retina. RGC soma size increases with retinal eccentricity (Dacey and Petersen, 1992; Yamada et al., 2001), indicating that similar functional roles may be carried out by cells of different sizes depending on their location within the retina. The greater loss of large RGCs in the visual streak, which we have observed, may indicate that alpha cells are more sensitive to the increased IOP than other RGC types. Similar to our findings in the porcine visual streak, a selective loss of large RGCs in the human and monkey fovea has been described during naturally occurring and experimentally induced glaucoma (Asai et al., 1987; Glovinsky et al., 1993).

Selective death of large RGCs within the mid-peripheral and peripheral retina was more difficult to evaluate, since the size of surviving RGC soma within these retinal regions increased after the period of elevation of IOP. Thus, in these regions the death of large and/or medium-sized RGCs is probably followed by a shift in cell size from small and medium RGCs to medium and large RGCs in response to the glaucomatous process. In the mid-periphery, we observed a significant loss in number of large RGCs despite the incorporation of previously medium-sized RGCs to this size category. This observation supports the hypothesis of a selective loss of large RGCs after glaucomatous damage.

The existence of a selective loss of large RGCs during glaucoma is nonetheless controversial (Kalloniatis et al., 1993; Graham et al., 1996; Morgan et al., 2000). Nevertheless, an increasing number of publications indicate that large RGCs are the main type affected during naturally occurring and experimentally induced glaucoma (Quigley et al., 1988; Glovinsky et al., 1991, 1993; Vickers et al., 1995; Anderson and O’Brien, 1997; Shou et al., 2003). The extensive loss of large diameter axons (Quigley et al., 1987, 1988) and post-mortem studies of the lateral geniculate nucleus of glaucoma patients or animals with experimentally induced glaucoma (Dandona et al., 1991; Chaturvedi et al., 1993; Weber et al., 2000) also point to a selective loss of large RGCs.

5. Concluding remarks

The main goal of the present study was to evaluate the pig eye as an experimental model of glaucoma. The pig eye was chosen since it is similar in many respects to the human eye. Moreover, the pig is an affordable animal and its use offers few ethical problems. Additionally, in contrast to previous studies involving the indirect analysis of monkey optic nerve fibres or geniculate neurons using Nissl staining, in the present study, we have directly analysed specifically labelled RGCs with Fluoro-Gold. The advantage of using this novel large mammal will be especially significant in experiments in which a large number of animals would be required.

Acknowledgements

Grants from The Glaucoma Foundation, European Community (QLK6-CT-2001-00385), Spanish Ministry of Science and Technology (BFI 2003-07177) and the University of the Basque Country (E-14887/2002; 15350/2003). MG holds an EC postdoctoral fellowship. JRE holds a predoctoral fellowship from the UPV and from the Jesus de Gangoiti Barrera Foundation MH holds a FPI predoctoral fellowship. We want to sincerely acknowledge Francisco Martín for his skillful help with the animals.

References


