FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2014/2015

Miguel Trigo Coimbra

Experimental animal models of induced intraocular hypertension

março, 2015

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Miguel Trigo Coimbra

Experimental animal models of induced intraocular hypertension

Mestrado Integrado em Medicina

Área: Fisiologia

Tipologia: Monografia

Trabalho efetuado sob a Orientação de:

Doutor Amândio António Rocha Dias de Sousa

Trabalho organizado de acordo com as normas da revista:

Experimental Eye Research

março, 2015

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Miguel Trigo Coimbra, abaixo assinado, nº mecanográfico 200903569, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 23/03/2015

Assinatura conforme cartão de identificação:

Miguel Trigo Coimbra

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

Miguel Trigo Coimbra

CARTÃO DE CIDADÃO OU PASSAPORTE (se estrangeiro)	E-MAIL	TELEFONE OU TELEMÓVEL
13633613	mimed09120@med.up.pt	+351 924124243

NÚMERO DE ESTUDANTE	DATA DE CONCLUSÃO
200903569	23 de Março de 2015

DESIGNAÇÃO DA ÁREA DO PROJECTO

Fisiologia

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Experimental animal models of induced intraocular hypertension

ORIENTADOR

Doutor Amândio António Rocha Dias de Sousa

COORIENTADOR (se aplicável)

———

É autorizada a reprodução integral desta ~~Dissertação~~/Monografia (riscar o que não interessa) para efeitos de investigação e de divulgação pedagógica, em programas e projectos coordenados pela FMUP.

Faculdade de Medicina da Universidade do Porto, 23/03/2015

Assinatura conforme cartão de identificação: Miguel Trigo Coimbra

Review

Experimental animal models of induced intraocular hypertension

M. Coimbra [a,*], A. Moleiro [a], A. Rocha-Sousa [a,b]

[a] Laboratory of Physiology, Faculty of Medicine, University of Porto, Portugal

[b] Department of Senses Organs, Faculty of Medicine, University of Porto, Portugal

* ***Correspondence****:*

Miguel Trigo Coimbra,

Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto

Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal.

Telephone: +351 924124243

E-mail: mimed09120@med.up.pt

Abstract

As the ethiologic and pathologic processes of glaucoma disease remain largely unknown, the benefit of animal models that effectively mimic the condition is crucial, and therefore they are increasing in popularity. The aim of this paper is to provide a descriptive summary for each of the included intraocular hypertensive methods and the results obtained, along with some of the advantages and limitations considering the models used.

A systematic revision of studies published between 1957 and 2014 in English in MEDLINE was performed. Search words used included: intraocular pressure, glaucoma, animal, model, laser photocoagulation, trabecular meshwork, episcleral vein, cauterization, ligation, microsphere, microbead, glycosaminoglycan, cellulose, steroid, cannulation, Morrison, monkey, rat, rabbit, cat, mouse, pig.

The models described promote impairment in the "conventional" aqueous humor drainage pathway with elevation of intraocular pressure, one major risk factor for glaucoma. The intraocular hypertensive mechanism was classified as pre-trabecular, if it simulates excessive aqueous humor inflow, or post-trabecular if a decreased aqueous humor outflow is obtained downstream from the trabecular meshwork, associated with vein congestion. Trabecular mechanisms induce obstruction or destruction of the trabecular meshwork itself. Many efficient and reproducible methods were performed in monkeys, rats, mice, rabbits, cats, pigs, sheep, bovine and ferrets.

Induced intraocular hypertension animal models are very promising in terms of a better understanding of the pathophysiologic processes of glaucoma, as well as in the discovery of novel therapies for this condition.

Keywords: Glaucoma, intraocular pressure, animal models.

1. Introduction

Elevated intraocular pressure (IOP) is nowadays accepted as the only known treatable major risk factor for glaucoma. It has been shown to affect the prevalence, incidence and development of this condition (Paulavičiūtė-Baikštienė et al., 2013), even though the underlying pathophysiologic mechanisms still remain uncertain (Wang et al., 2012). Nevertheless, changes in human glaucomatous eyes have been recently described in the trabecular meshwork, including excessive production of extracellular matrix by increased TGF-β2, impaired phagocytosis and increased contractility of trabecular meshwork cells. These changes lead to partial obliteration of the spaces between collagen beams, increasing the resistance to aqueous outflow, and ultimately increasing IOP (Paulavičiūtė-Baikštienė et al., 2013).

Animal models over the decades have proven to be crucial to understanding numerous diseases. Indeed, in terms of glaucoma research, a wide variety of animals of different species have been employed, and researchers took into account the animal size, cost and the amount of morphologic and physiologic overlap with human eye characteristics.

The intraocular hypertensive animal models considered to be more suitable to study glaucoma neuropathy should induce sustained moderately high IOP levels during a reasonable period. These models allow examination of both the precise onset and the progression of pathological changes of glaucomatous disease in a controlled and reproducible way (Vecino and Sharma, 2011). The pathophysiologic changes observed in the retinal ganglion cell (RGC) layer should be similar to those in human individuals, and optic disk cupping should be observed. They are also important to evaluate novel therapies in an organized manner (Samsel et al., 2011).

Genetic animal models of glaucoma impose practical difficulties to many researchers worldwide, including availability and cost, and they were not included in this review. Normal tension glaucoma models were also not included.

2. Methods

A systematic revision of studies published between 1957 and 2014 in English in MEDLINE was performed. Search words used included: intraocular pressure, glaucoma, animal, model, laser photocoagulation, trabecular meshwork, episcleral vein, cauterization, ligation, microsphere, microbead, glycosaminoglycan, cellulose, steroid, cannulation, Morrison, monkey, rat, rabbit, cat, mouse, pig.

3. The aqueous humor outflow pathway

In humans, the aqueous humor is produced by the ciliary epithelium at the ciliary processes in the *pars plicata* of the ciliary body, and is secreted into the posterior chamber. It flows between the lens and the iris, through the pupil and into the anterior chamber. From the anterior chamber angle, the aqueous humor is drained by a "conventional" pathway through the trabecular meshwork and into the Schlemm's canal, draining collector channels, radial aqueous veins and episcleral veins by this order. However, it can also be drained by a "non-conventional" pathway through the ciliary muscle anteriorly, uveal meshwork, suprachoroidal space and finally the sclera. Some authors consider this pathway to be analogous to lymphatic drainage (Johnson and Erickson, 2000), it decreases with age and is responsible for approximately 40 to 50% of aqueous humor drainage. However, it is independent from IOP level variation, and when aqueous humor production surpasses its drainage through the "conventional" pathway, an elevation of IOP occurs (Goel et al., 2010).

The models described in this section are aimed at artificially impairing the "conventional" aqueous humor drainage pathway with elevation of IOP levels. For reasons of convenience, we decided to categorize the models according to the site of intervention mechanism relatively to the trabecular meshwork, as listed in table 1.

3.1. Chymotrypsin Injection

The long-term ocular hypertension induced by alpha-chymotrypsin is believed to result from an increased aqueous humor inflow by disruption of the blood-aqueous barrier, rather than trabecular blockage by lysed zonular material (Melena et al. 1999; Chee and Hamasaki, 1971; Anderson, 1971).

3.1.1. Monkey

Several authors described cupping of the optic disk and atrophy of the optic nerve as early as 3 days of sustained rise in IOP after injecting 75 and 750 units (respectively) of chymoptrypsin in the posterior chamber of monkeys (Kalvin et al., 1966; Lessell and Kuwabara, 1969). However, the subjects with higher doses of chymotrypsin often underwent corneal perforation.

3.1.2. Rabbit

Based on many authors (Sears and Sears, 1974; Gelatt, 1977; Vareilles et al., 1979), Fernandez-Durango et al. (1991) injected unilaterally 0.86 mg of α-chymotrypsin diluted in 0.13 mL of saline solution into the posterior chamber of New Zealand albino rabbits. They found increased IOP as soon as 3 days in 82 % of the animals, which maintained during 40 days, with mean values of 28.4 mmHg in treated eyes compared to 13.1 mmHg in contralateral control

eyes. This is a well-established chronic intraocular hypertensive model, with relevant similarities to human glaucoma (Percicot et al., 1996).

3.2. Manometric Intraocular cannulation

3.2.1. Monkey

Anderson and Hendrickson (1974) originally describe a method in which they manometrically control the IOP in owl monkeys. A 27 gauge needle is inserted obliquely through the peripheral cornea and into the posterior chamber. It is connected to an external saline container, which content is varied according to the systemic blood pressure, measured by femoral artery cannulation, in order to maintain a constant perfusion pressure (PP), measured as the difference between mean arterial pressure and IOP, during eight hours.

The authors observed that at slightly elevated levels of IOP (PP of 60 mmHg) there was a partial obstruction of axoplasmic transport, which became more obvious at moderate levels of IOP (PP of 35 to 45 mmHg). Severe obstruction to axonal transport was achieved at higher IOP levels (PP = 25 mmHg) with greater axonal dilation. However, with a PP under 25 mmHg, there was a complete absence of axoplasmic transport in the ganglion cells. According to Anderson and Davis (1975), it is at a perfusion pressure under 15 mmHg that most permanent retinal changes occur, namely partial necrosis of iris, stroma and ciliary processes, macroscopic lesions around the disc and in the retinal periphery, possibly due to a nonischemic pressure-induced mechanism in chronic glaucoma.

3.2.2. Cat

In adult cats Grehn et al. (1984) inserted a Ringer solution injecting cannula (20 U/mL) and a pressure transducing cannula in the anterior chamber through the limbus. The perfusion pressure was calculated as the difference between mean blood pressure and IOP.

The authors confirmed that PP is the main ischemic factor, since animals with higher mean blood pressure also tolerate higher IOP levels, and they observed that total suppression of retinal neurons transmission is only observed when PP reaches critical levels below 20 mmHg in cats. The authors also mention that short-term IOP elevation by cannulation at critical PP levels preferentially causes functional retinal impairment by anoxia and can be reversible up to 100 minutes, whereas a long-term approach with moderate PP levels (50 mmHg) causes mechanically induced optic disk excavation.

3.2.3. Rat

Identically, Büchi et al. (1991) developed a rat model of anterior chamber cannulation raising the intraocular pressure (IOP) to 110 mmHg during a period of 120 minutes, and suggested a replacement of normal saline solution with a 5% dextrose solution, which proved to decrease the frequency of retinal ischemic injury after 120 minutes.

3.3. Laser Photocoagulation of the Trabecular Meshwork

In theory, partial scarring of the trabecular meshwork and obliteration of the Schlemm's canal, as observed histologically by Gaasterland and Kupfer (1974), should induce significant resistance to aqueous humor outflow and increase IOP in a sustained manner.

3.3.1. Monkey

Gaasterland and Kupfer (1974) used a circumferential argon laser photocoagulation model on rhesus monkeys, with approximately two hundred laser applications aimed at scarring the middle of the trabecular meshwork in the anterior chamber of both eyes. A 50 μm beam diameter was used, ranging from 0.2 to 0.5 seconds duration with a power of 0.4 to 0.8 Watts. Two to four treatments were applied. Up to 12 weeks later, 70% had successfully elevated IOP from baseline values of 11 – 14 mmHg to 24 – 50 mmHg. Optic disc cupping was present in 60% and outflow facility impairment in 100%. Thinning of the nerve fiber layer was also observed.

Based on Gaasterland and Kupfer (1974), Quigley and Hohman (1983) concluded that a single laser treatment with 50 Joule of energy was more efficient, with exposure duration between 0.5 and 1.0 seconds.

A nonhuman primate model of laser trabecular photocoagulation has many anatomical and clinical similarities with human primary open angle glaucoma, and develops over the course of months instead of years. The mechanical stress on load-bearing tissues, such as the sclera and the lamina cribrosa, has been associated to loss of retinal and foveal ganglion cells (Roberts et al., 2010; Glovinsky et al., 1991, 1993), and also impairment in optic nerve head microcirculation (Wang et al., 2012).

3.3.2. Rat

Ueda et al. (1998) originally described a laser photocoagulation method in Wistar rats. Firstly, they injected 0.05 mL of India ink unilaterally into the anterior chamber. Direct argon laser photocoagulation is performed to the pigmented target in the trabecular meshwork, using 60 to 100 laser spots with 0.5 mm width, 250 mW of power and 0.2 seconds duration.

After 3 laser treatments, mean IOP in 6 out of 7 eyes was higher than 25 mmHg at the fourth week. Maintaining IOP elevated required 10 to 30 laser burns if IOP dropped to a value around 20 mmHg, or 60 to 100 laser burns if IOP dropped below 15 mmHg. Cavernous degeneration of the optic nerve head and reduced thickness of the nerve fiber layer were observed.

The authors believe the chronic IOP elevation is caused by peripheral anterior synechia, which led to a structural angle closure, and also reduced intertrabecular spaces, with accumulation of extracellular matrix and macrophages containing most carbon particles. However, they admit structural differences between the lamina cribrosa of rodents and primates that should be considered when studying the pathogenesis of nerve damage in glaucoma.

3.3.3. Mouse

Aihara et al. (2003) have explored the unilateral translimbal laser photocoagulation of the trabecular meshwork approach described elsewhere (Levkovitch-Verbin et al., 2002) in black Swiss mice, with a laser power of 200 mW, 0.05 seconds duration and 200 µm spot diameter. For optimization of this model, the authors induced unilateral mydriasis and flattening of the anterior chamber by aqueous fluid aspiration. During the first 4 weeks, a success rate of 64% was noted, with a mean IOP increase of 30% compared to control values. However, after 12 weeks, only 34% maintained elevated IOP. Some treated eyes developed corneal edema, corneal opacity and cataract. This method was reproduced later with a significant decrease in the optic nerve cross-sectional area (28.5%), mean axon density (57.8%) and total number of axons (63.1%) in treated eyes (Mabuchi et al., 2003).

Therefore, the authors believe that flattening of the anterior chamber leading to angle closure contributes largely to a satisfyingly sustained IOP elevation after one single intervention. However, the reproducibility of this method is not adequate, since there was a high variability in IOP levels. Besides, mice are relatively small and difficult to handle (Aihara et al., 2003).

3.4. Microspheres

Quigley et al. (1979) originally described a method of trabecular meshwork obstruction in rabbits and monkeys using fixed degenerating red blood cells which possess a rigid outer membrane and a diameter of approximately 7.5 µm. This is analogous to the modern intraocular microsphere obstruction mechanism, which is described as occurring preferentially at the interlaminar collagen spaces in the inner wall of Schlemm's canal (Inomata et al., 1972). Some authors suggest an inflammatory reaction at the trabecular meshwork might also contribute to the reduction in aqueous humor outflow (Weber and Zelenak, 2001).

3.4.1. Monkey

Weber and Zelenak (2001) injected 50 µL through the cornea and into the anterior chamber of a 0.15 M NaCl aqueous solution containing approximately 2 x 10^5 latex microspheres with a diameter of 10 µm and fluorescein dye. A maximum of 2 injections per week were given whenever IOP dropped below 30 mmHg. The injection time was lengthened between 1 and 2 minutes to avoid abnormal peaks in IOP. The microsphere deposition was monitored non-invasively using Zeiss slit lamp and gonioscopy with a cobalt filter. IOP elevation was successful in all animals, and a mean IOP value of 36.5 mmHg in the hypertensive eye and 17.3 mmHg in the control eye were obtained during 36 months. The optic cup-to-disk ratio increased progressively from 0.2 to 0.8, and a 70 % decrease of optic nerve axons was observed. The authors defend this method is simple and inexpensive.

They argue that varying the concentrations and volumes of microsphere solutions, as well as the number of treatments, is correlated to the extent and duration of increased IOP.

3.4.2. Rat

Sappington et al. (2010) described an intracameral injection in brown Norway Rats of a solution containing 10^6 polystyrene microbeads per mL, with a diameter of 15 µm and bound to a 488nm-cromophore. The contralateral control eye received an identical volume of sterile physiological saline solution. A glass micropipette with a diameter of 100 µm was placed approximately 3 mm within the *ora serrata* over the cornea, and 5 µL were injected at a rate of 1 µL per second. A range of mean IOP elevation from 21 to 34% was recorded during 2 weeks, compared to the control eyes. They witnessed a direct correlation between the microbead injection volume and the mean IOP value. However, IOP variability is lower at higher microbead volumes.

3.4.3. Mouse

Sappington et al. (2010) described the same method for C57BL/6 mice, with a lower polystyrene microbead injection volume of 1 µL. Overall, mice had a more regular elevation in mean IOP of 30% during 3 weeks.

A modified version was described by Cone et al. (2012) in CD1 albino and C57BL/6 pigmented mice. The authors tested for a "4+1" protocol, in which they injected 2 µL of 6 µm beads (at $3x10^6$ beads per µL), 2 µL of 1 µm beads (at $1.5x10^7$ beads per µL) and 1 µL of viscoelastic solution containing 10 mg/mL of sodium hyaluronate, by this order. The mice reached an average IOP difference of 6.1 mmHg and a mean axon loss of 36% up to 6 weeks, which was higher for CD1 mice.

3.5. Magnetic microspheres

These microspheres are equally easily injectable into the anterior chamber, and have the advantage of being preferentially directed to the iridocorneal angle using magnetic fields, obstructing the trabecular meshwork more efficiently and improving subsequent fundus examinations (Samsel et al., 2011).

3.5.1. Rat

In Brown Norway Rats, Samsel et al. (2011) injected between 10 and 20 µL of a previously agitated sterile balanced salt solution containing 30 mg/mL iron-magnetic microspheres, with a diameter of 5 µm, unilaterally into the anterior chamber. Meanwhile, a small handheld magnet (0.45 Tesla) was positioned externally from the iridocorneal angle. In average, 12.8 days with a mean sustained elevation of 5.8 mmHg in IOP were obtained. A mean RGC loss of 36.4 % was also observed. The authors conclude that this method is simple, cost-effective and easily reproducible, either in single or in chronic weekly injections. Furthermore, it optimizes trabecular obstruction since a smaller number of microsphere injections (up to three) were needed for successfully increasing IOP, compared to other microsphere models.

3.6. Conjunctival Cells

3.6.1. Ferret

Fujishiro et al. (2014) have developed an IOP elevation model in adult ferrets by unilateral injection of cultured conjunctival cells in the anterior chamber using a 32 gauge needle. Average IOP in treated eyes was 42.8 mmHg, compared to 14.1 mmHg in the contralateral eyes, during 13 weeks after a single injection. Histologically, the iridocorneal angle was covered with proliferated cells obstructing the trabecular spaces, there was optic disk cupping and degeneration of optic nerve axons. The authors argue that ferrets have a more developed binocular vision compared to rodents, therefore more closely related to humans when assessing spatial patterns of degeneration in the central visual system in glaucoma. However, inflammation after the cell injection was common and IOP levels resembled closed-angle glaucoma, therefore care should be taken not to induce retinal ischemia.

3.7. Viscoelastic Substances

3.7.1. Glycosaminoglycans

Hyaluronic acid is the most abundant GAG in the trabecular meshwork (Acott et al., 1985). It is not an antigenic or inflammatory substance, no species variations have been described and it can form viscous dilute solutions due to its water-binding capacity, thus being suitable for eye

surgery (Lanza, 2014). Knepper et al. (1996) observed an abnormal accumulation of mucopolysaccharides at the trabecular meshwork of primary open glaucoma patients, with reduced amount of hyaluronic acid and increased chondroitin sulfates. Benozzi et al. (2000) suggested that decreased IOP and increased aqueous humor outflow could be allowed by stimulating hyaluronidase activity.

3.7.1.1. Monkey

Schubert et al. (1984) injected 0.22mL of various solutions containing sodium-hyaluronate at 1% with different molecular weights and into the anterior chamber of owl monkeys, in exchange for the same volume of aqueous humor. They inserted a 27-Gauge needle through the cornea 1 mm proximal to the limbus. Most solutions caused consistently increased IOP during the first 4 to 7 hours, which returned to normal within 24 hours. The authors witnessed a sooner and higher peak of 60 mmHg in IOP in low viscosity solutions, in which they concluded that the amount of exogenous sodium-hyaluronate turnover from the anterior chamber passing through the outflow channels is higher, since they mix faster with the pre-existing aqueous humor.

3.7.1.2. Rabbit

In New Zealand White rabbits, Harooni et al. (1998) unilaterally injected 0.10 mL of viscous sodium hyaluronate solution and 0.05 mL of a balanced saline solution, in exchange for 0.15 mL of aqueous humor. They tested sodium hyaluronate at 1% (Healon®), 1.4% (Healon GV®) and 3% with additional chondroitin sulphate at 4% (Viscoat®) during 72 hours. There was a significant increase in IOP with values between 25 and 35 mmHg during the initial 24 hours for all viscosities. Equi et al. (1997) add that IOP levels are directly correlated to the polymer size of hyaluronate. When Harooni et al. (1998) replaced the saline solution for hyaluronidase in the contralateral eye, however, only a slight and not significant decrease of IOP was observed.

3.7.1.3. Rat

Benozzi et al. (2002) used a 30 Gauge needle to inject 25 µL of a saline solution containing hyaluronic acid at 1% (10 mg/mL) unilaterally into the anterior chamber of Wistar rats. In a single injection, a mean IOP value of 20.8 mmHg was observed (versus 12 mmHg in the contralateral control eye), and remained significantly higher until the 8th day. With chronic administration of hyaluronic acid, IOP in treated eyes was approximately 20.6 mmHg during 10 weeks. The authors believe the rat acute intraocular hypertensive model lasts longer than the rabbit and the monkey acute models (24 hours) since the intracameral concentration of hyaluronic acid achieved is higher in a narrower anterior chamber. They also defend their chronic model is effective, inexpensive, easy to perform and a large number of experimental rodents can be used.

3.7.2. Cellulose derivatives

Cellulose derivatives present viscoelastic properties. They are not toxic or inflammatory and they mechanically decrease the outflow facility (Zhu and Cai, 1992).

3.7.2.1. Rabbit

Zhu and Cai (1992) injected a single dose of methylcellulose (MC) at 1 or 2%, hydroxypropyl methylcellulose (HPMC) at 2 % and sodium carboxymethylcellulose (SCMC) at 2% into the anterior chamber of pigmented rabbits, by aqueous humor exchange of 0.25 mL. The rabbits which received MC at 1 or 2 % had a similar mean IOP of 31.4 and 33.3 mmHg, respectively, compared to 19.6 mmHg control levels, during approximately 16 days. SCMC at 2 % had a similar mean elevated IOP of 32.0 mmHg, but for a shorter period (8 days). However, IOP levels in the group with HPMC at 2 % were not significantly high, which confirms previous clinical findings (Liesegang et al., 1986). Törngren et al. (2000) achieved a peak in IOP of approximately 47 mmHg between 2 and 4 hours after injecting a larger volume of 50 µl of HPMC at 2% in rabbits. Still, IOP decreased for non-significant levels 12 hours later.

Zhu and Cai (1992) repeated the injection of cellulose derivatives if IOP fell below 30 mmHg. During 8 weeks, they registered mean IOP levels of 36.5 mmHg, 35.7 mmHg and 36.5 mmHg, for MC at 1% and 2 % and SCMC at 2 %, respectively.

Overall, they concluded that IOP elevation by single injections of cellulose derivatives lasted longer than other methods, with fewer side effects. Pronounced optic disk cupping, hyperemia, corneal edema and sustained high IOP levels make the rabbit groups with multiple injections of MC at 1 and 2 % promising for the study of human chronic open-angle glaucoma (Zhu and Cai, 1992).

3.8. Corticosteroids

McLean et al. observed the first steroid-induced intraocular hypertensive effect with systemic adrenocorticotropic hormone (Gordon et al., 1951). Later, François (1977) described how steroids inhibit the lysossomal hyaluronidase release, preventing its effect on hyaluronates and increasing the amount of aggregated mucopolysaccharides in the extracellular matrix of the trabecular meshwork. In addition, trabecular meshwork cells suffer enlargement of the nucleus and cytosol organelles and also reduced phagocytic properties in response to dexamethasone. All these changes contribute to thickened trabecular and juxtacanalicular tissues and decreased intertrabecular spaces, thus leading to an increased resistance to aqueous outflow (Clark and Wordinger, 2009; Wilson et al., 1993).

3.8.1. Rabbit

Bonomi et al. (1978) believed that the chronic effect of high local corticosteroid doses spreading systemically caused organic side effects associated with heavy weight loss, considerable IOP fluctuations and often death to the subjects. Therefore, they proposed a model of 3 unilateral subconjunctival injections in rabbits, once per week, with 4mg of a repository betamethasone preparation containing hydroxiethylcellulose. From baseline IOP levels of 18 mmHg, a mean peak of 27 mmHg was reached at the third week in 96% of the animals, but the contralateral control eye also reached a peak of 22 mmHg. Nevertheless, significant elevated IOP levels were maintained until 5 weeks. An improved mortality rate of 3.12% was also described.

Knepper et al. (1978), however, have witnessed differently. With 1 drop of topical dexamethasone at 0.1% every 6 hours, they concluded that at 4 weeks only young rabbits aged between 8 and 10 weeks were responsive, possibly due to an age-related difference in the ratio of keratan sulfate to uranic acid containing glycosaminoglycans in the trabecular meshwork. It represents a clear contrast with human glaucoma, which is progressively induced, preferably in older individuals.

3.8.2. Cat

Zhan et al. (1992) applied a 10 μL solution of dexamethasone sodium phosphate (at 1.0%) to the cornea 3 times daily during a month to normotensive cats. However, only a group of cats which underwent an interval of 7 days without treatment and repeated the dexamethasone sodium phosphate (1%) twice daily for another month showed a fairly significant IOP elevation. When applying a solution containing 10 μL of 1% prednisolone acetate topically twice a day with a 3 to 5 minute interval to ocular normotensive cats, a slight but significant increase in IOP was observed after 22 days and up to 60 days. Increasing the frequency of administration did not further significantly increase the IOP. The authors emphasize that the feline eye resembles the human eye at a greater extent in terms of intraocular corticosteroid target sites than the rabbit eye.

3.8.3. Rat

Young Wistar rats were given topical ocular dexamethasone 4 times daily for 4 weeks (Sawaguchi et al., 2005). IOP was increased slightly but significantly since the second week of treatment.

3.8.4. Bovine

Gerometta et al. (2004) provided 12 cows with unilateral topical ocular prednisolone acetate drops at 1% initially and 0.5% after the first week, 3 times daily during 49 days. There was a significant IOP variation from 7 to 15 mmHg between hypertensive and control eyes and up to 10 weeks.

3.8.5. Sheep

18 sheep received unilateral topical ocular prednisolone acetate (0.5%) drops 3 times daily during 4 weeks (Gerometta et al., 2009). All developed increased mean IOP values of 23 mmHg in the second week and 27.5 mmHg in the third week, compared to contralateral values of 11.2 to 11.7 mmHg. Therefore, they concluded that the results in sheep and bovine steroid models are more enticing than in other animals.

3.9. Laser Photocoagulation of limbal venous plexus and episcleral veins

3.9.1. Rat

WoldeMussie et al. (2001) used blue-green Argon laser photocoagulation on the limbal venous plexus and episcleral veins within 0.5 to 0.8 mm from the limbus. 130 to 150 laser burns, with 0.05mm width, a power of 1 Watt and 0.2 seconds duration were used. A second treatment was performed 1 week later. An increase of 60% and 100% from baseline IOP values was accomplished after the first and second treatments, respectively, and maintained significantly high for 2 months. Considering a slowed RGC loss rate from 12% to 2% per week between the third week and two months, the authors defend a direct correlation with IOP levels during this period.

3.9.2. Mouse

Identically, Gross et al. (2003) described a laser photocoagulation model for C57BL/6J mice. Episcleral and limbal veins were photocoagulated within 1 mm from the limbus, using a laser with 50 µm width, 0.1 seconds duration and a power between 80 and 110 mW. At 4 weeks, 90% had a mean IOP value of 20 mmHg versus baseline values of 13 mmHg, and a RGC loss of 22.4%. However, the C57BL/6J mouse strain used develops anterior segment anatomic abnormalities that might interfere with IOP values.

3.10. Episcleral Vein Cauterization

In this model, IOP elevation is likely to result from vein congestion and reduction of aqueous humor outflow combined (Naskar et al., 2002).

3.10.1. Rats

Shareef et al. (1995) describe a model of unilateral cauterization of a variable number of deep episcleral veins. After dissecting the conjunctiva at the limbal periphery and exposing the extraocular muscles, a suture underneath would anchor them and reveal the adjacent deep episcleral veins. Once the vein is visually detached from the overlying conjunctiva and muscle, an ophthalmic cautery is applied to the episcleral trunk to fully interrupt the drainage (Shareef et al., 1995). Alternatively, Sharma (2003) argue that cauterization to the junction of aqueous-containing radial veins and ciliary veins is also effective.

Shareef et al. (1995) observed that if 2 or more veins were cauterized, a rise of at least 90% was observed in IOP. However, after 1 week, IOP returned to values slightly higher than baseline. In contrast, Naskar et al. (2002) cauterized 2 episcleral veins in adult Sprague-Dawley rats and observed a 1.6 fold increase in IOP levels up to 3 months. When Shareef et al. (1995) cauterized 3 veins, IOP increased from 13.2 mmHg to 53 mmHg, stabilizing at 29 mmHg after 2 months. Little to none decompensation symptoms were found in this group. When all four veins were cauterized, a stable raised IOP level of 60 mmHg was maintained until 1.5 weeks, but most eyes showed proptosis, exposure keratopathy, corneal edema and corneal endothelial malfunction (Shareef et al., 1995), and Sharma (2003) observed a high risk of necrosis of the eye within 1 week.

This method is reproducible and excellent for the study of primary open angle glaucoma, as well as to test for possible neuroprotective agents (Naskar et al., 2002; Shareef et al., 1995; Sharma, 2003). However, practice is necessary (Sharma, 2003), since initial trials only led to a success rate of 25%, compared to 85% later on. They also observed that leaking from the cauterization site was associated with neovascularization in up to 15%, and hence they suggest ligating the vein before cauterization.

3.10.2. Pig

Ruiz-Ederra et al. (2005) cauterized unilaterally 3 episcleral veins of adult pigs. Since the third week and during 21 weeks, an increased mean IOP value of 20.8 mmHg was witnessed (versus control levels of 15.6 mmHg). The significant RGC death in the mid-peripheral and peripheral retina observed resembles the human pattern of RGC degeneration in glaucoma.

3.10.3. Mouse

According to Aihara et al. (2003) the mouse eye anatomy is too small and the outflow vessels form a thin plexus rather than largely visible episcleral veins. Moreover, the mouse sclera is too thin and a high incidence of perforation may result. In fact, Ruiz-Ederra and

Verkman (2006) excluded 12 out of 35 mice due to scleral complications and episcleral vein leakage from cauterization of 3 deep episcleral veins. Nonetheless, they achieved an IOP elevation success rate of 87% and a maximum peak of 28 mmHg up to nine days after the procedure. The results were variable, but 94% showed significantly increased IOP until 4 weeks. They also observed aqueous outflow resistance 2.5 times higher than in control eyes, and a RGC loss of 20%.

3.11. Episcleral Vein Ligation

3.11.1. Rats

Yu et al. (2006) described a method of unilateral ligation of 3 deep episcleral veins after their exposure in the respective eye quadrants, by dissecting through the overlying conjunctiva and Tenon's capsule. A 10-0 nylon was used. 40.8% of treated eyes had a mean IOP value of 24.7 mmHg for 7 months. In 59.2%, however, there was a decrease below 25 mmHg until 4 weeks, and another intervention with ligation of collateral neovasculature was performed. Optic disk escavation and a RGC loss of 1.3% weekly were also reported.

3.11.2 Rabbit

As originally described by Huggert (1957), Zhu and Cai (1992) observed that the ligation of three vortex veins in adult pigmented rabbits induced a mild mean value of 27.1 mmHg (versus baseline levels of 19 mmHg) during approximately 11 days only. These results could be explained by collateral circulation and an enlargement up to three times higher of the unoperated vortex vein. Retinal hemorrhage was often found, while hyperemia and corneal edema were sometimes present. The authors concluded this method is not adequate for a chronically induced rise in IOP.

3.12. Saline Injection in Episcleral Veins

3.12.1. Rat

The limbal vasculature, Schlemm's canal and trabecular meshwork of the rat eye are mostly analogous to primates, and a "conventional route" in rats is identically responsible for only part of the aqueous humor outflow (Morrison et al., 1995).

Morrison et al. (1997) induced sclerosis of episcleral veins in Brown Norway rats. After a lateral canthotomy and the dissection of the conjunctiva with exposure of one radial aqueous vein, 50 µL of hypertonic saline solution at 1.65 or 1.75 M are injected causing whitening of the vessel. If IOP fails to rise significantly until 2 weeks, a second cannulation is performed in an opposite radial aqueous vein. A success rate of 80% was achieved. IOP increase was variable

between 7 and 28 mmHg during 200 days. However, inflammation occurred often, and sometimes sclerosis of the trabecular meshwork with anterior synechia was visible at the iridocorneal angle. They witnessed an increased superior temporal susceptibility to axonal degeneration and a pressure-related deposition of extracellular matrix components in the optic nerve head fibers, both analogous to human glaucomatous eyes.

3.13. External oculopression

3.13.1. Rat

Sellés-Navarro et al. (1996) described a mechanical method of increasing the IOP by external oculopression. In Sprague-Dawley rats, two 6-0 silk sutures were placed unilaterally around the bulbar conjunctiva on either sides of the corneoscleral limbus and were pulled on opposite directions. The eye was kept above systolic arterial pressure levels during the ischemic period. Interruption of blood flow duration varied from 30 to 120 minutes.

During 30 days, only periods of transient ischemia longer than 45 minutes induced retinal RGC death, which occurred as early as 3 hours and became more severe with longer ischemic intervals. Periods of ischemia of at least 90 minutes cause the death of approximately 50% of the RGC population after 5 days and 95% after 30 days. Mild edema of the conjunctiva and cornea were commonly present, and sometimes retinal or vitreous hemorrhages were also found.

3.14. Laser Photocoagulation of trabecular meshwork and episcleral veins

3.14.1. Rat

In Wistar rats, Levkovitch-Verbin et al. (2002) combined 60 to 80 unilateral laser deliveries to the trabecular meshwork, mostly through the cornea, and also 15 to 20 laser spots to episcleral veins. Using a diode laser at 532 nm wave-length through a slit-lamp mechanism, they determined that a laser power of 0.4 Watt with 0.2 second duration is more efficient, witnessing a mean IOP of 25.5 mmHg versus 19.8 mmHg in control eyes during 9 weeks. Since 75% of the animals returned to baseline IOP levels mostly after 3 weeks, they required another intervention. The authors argue that the extent of RGC loss in the retina is associated with higher IOP levels. They concluded that their model is still expensive, yet reproducible, relatively simple to perform and has many similarities with human glaucoma.

4. Conclusion

This paper is an attempt to summarize most intraocular hypertension animal models currently used by the scientific community, especially for a better insight into glaucoma processes (Table 1).

Recent investigation in glaucoma therapy is directed to the risk factors instead of the etiology and pathophysiology of the disease, and current clinical practice focuses on the reduction of aqueous humor formation or increasing the outflow, and thus lowering IOP (Paulavičiūtė-Baikštienė et al., 2013; Cioffi,2011). As such, establishing an effective and reproducible induced intraocular hypertension animal model is essential. Not forgetting that the pathophysiologic changes witnessed in these models should ideally be similar to those found in humans, comparison must be careful and generalizing from a certain animal and method should be avoided. In the meanwhile, as more technology is being developed in this field, we hope to achieve considerable progresses in prevention as well as treatment of glaucoma.

Level of aqueous flow modification	Procedure	Animal	Author
Pre-trabecular [a]	Chymotrypsin	Monkey	Kalvin et al. (1966); Lessell and Kuwabara (1969)
		Rabbit	Fernandez-Durango et al. (1991); Percicot et al. (1996)
	Manometric Intraocular Cannulation	Monkey	Anderson and Hendrickson (1974) Anderson and Davis (1975)
		Cat	Grehn et al. (1984)
		Rat	Büchi et al. (1991)
Trabecular [b]	Laser Photocoagulation of trabecular meshwork	Monkey	Gaasterland and Kupfer (1974) Quigley and Hohman (1983)
		Rat	Ueda et al. (1998)
		Mouse	Aihara et al. (2003) Mabuchi et al. (2003)
	Latex Microspheres	Monkey	Weber and Zelenak (2001)
	Polystyrene Microspheres	Rat	Sappington et al. (2010)
		Mouse	Sappington et al. (2010) Cone et al. (2012)
	Magnetic microspheres	Rat	Samsel et al. (2011)
	Conjunctival Cells	Ferret	Fujishiro et al. (2014)
	Glycosaminoglycans	Monkey	Schubert et al. (1984)
		Rabbit	Harooni et al. (1998) Equi et al. (1997)
		Rat	Benozzi et al. (2002)
	Cellulose derivatives	Rabbit	Zhu and Cai (1992) Törngren et al. (2000)
	Corticosteroids	Rabbit	Bonomi et al. (1978) Knepper et al. (1978)
		Cat	Zhan et al. (1992)
		Rat	Sawaguchi et al. (2005)
		Bovine	Gerometta et al. (2004)
		Sheep	Gerometta et al. (2009)

Post-trabecular [c]	Laser Photocoagulation of limbal venous plexus and episcleral veins	Rat	WoldeMussie et al. (2001)
		Mouse	Gross et al. (2003)
	Episcleral Vein Cauterization	Rat	Shareef et al. (1995) Naskar et al. (2002) Sharma (2003)
		Pig	Ruiz-Ederra et al. (2005)
		Mouse	Ruiz-Ederra and Verkman (2006)
	Episcleral Vein Ligation	Rat	Yu et al. (2006)
		Rabbit	Huggert (1957) Zhu and Cai (1992)
	Saline Injection in Episcleral Veins	Rat	Morrison et al. (1997)
	External oculopression	Rat	Sellés-Navarro et al. (1996)
Trabecular [b] and Post-Trabecular [c]	Laser Photocoagulation of trabecular meshwork and episcleral veins	Rat	Levkovitch-Verbin et al. (2002)

Table 1: List of intraocular hypertensive animal models categorized according to the level at which the impairment in aqueous humor flow occurs. [a]Pre-trabecular refers to models which simulate mostly an unbalanced increase in aqueous humor inflow. [b]Trabecular refers to models which induce obstruction or destruction of the trabecular meshwork. [c]Post-trabecular indicates models which preferentially decrease aqueous humor outflow through the "conventional" pathway downstream from the trabecular meshwork, causing vein congestion.

References

Acott, T.S., Westcott, M., Passo, M.S., Van, Buskirk, E.M., 1985. Trabecular meshwork glycosaminoglycans in human and cynomolgus monkey eye. Invest. Ophfhalmol. Vis. Sci. 26, 1320-q.

Aihara, M., Lindsey, J.D., Weinreb, R.N., 2003. Experimental mouse ocular hypertension: establishment of the model. Invest. Ophthalmol. Vis. Sci. 44(10): 4314-20.

Anderson, D.R., 1971. Experimental alpha chymotrypsin glaucoma studied by scanning electron microscopy. Am. J. Ophthalmol. 71(2): 470-6.

Anderson, D.R., Davis, E.B., 1975. Sensitivities of ocular tissues to acute pressure-induced ischemia. Arch. Ophthalmol. 93(4): 267-74.

Anderson, D.R., Hendrickson, A., 1974. Effect of intraocular pressure on rapid axoplasmic transport in monkey optic nerve. Invest. Ophthalmol. 13(10): 771-83.

Benozzi, J., Jaliffa, C.O., Firpo, Lacoste, F., Llomovatte, D.W., Keller, Sarmiento, M.I., Rosenstein, R.E., 2000. Effect of brimonidine on rabbit trabecular meshwork hyaluronidase activity. Invest. Ophthalmol. Vis. Sci. 41(8): 2268-72.

Benozzi, J., Nahum, L.P., Campanelli, J.L., Rosenstein, R.E., 2002. Effect of hyaluronic acid on intraocular pressure in rats. Invest. Ophthalmol. Vis. Sci. 43(7): 2196-200.

Bonomi, L., Perfetti, S., Noya, E., Bellucci, R., Tomazzoli, L., 1978. Experimental corticosteroid ocular hypertension in the rabbit. Albrecht. Von. Graefes. Arch. Klin. Exp. Ophthalmol. 209, 73-82.

Büchi, E.R., Suivaizdis, I., Fu, J., 1991. Pressure-induced retinal ischemia in rats: an experimental model for quantitative study. Ophthalmologica. 203(3): 138-47.

Chee, P., Hamasaki, D.I., 1971. The basis for chymotrypsin-induced glaucoma. Arch. Ophthalmol. 85(1): 103-6.

Cioffi, G.A. (editor), 2011. 2011-2012 Basic and clinical science course (BCSC). Section 10: Glaucoma. American Academy of Ophthalmology, San Francisco.

Clark, A.F., Wordinger, R.J., 2009. The role of steroids in outflow resistance. Exp. Eye Res. 88(4): 752-9. Review.

Cone, F.E., Steinhart, M.R., Oglesby, E.N., Kalesnykas, G., Pease, M.E., Quigley, H.A., 2012. The effects of anesthesia, mouse strain and age on intraocular pressure and an improved murine model of experimental glaucoma. Exp. Eye Res. 99: 27-35.

Equi, R.A., Jumper, M., Cha C., Stern, R., Schwartz D.M. 1997. Hyaluronan polymer size modulates intraocular pressure. J Ocul Pharmacol Ther. 13: 289–295.

Fernandez-Durango, R., Ramirez, J.M., Trivino, A., Sanchez, D., Paraiso, P., Garcia De Lacoba, M., Ramirez, A., Salazar, J.J., Fernandez-Cruz, A., Gutkowska, J., 1991. Experimental glaucoma significantly decreases atrial natriuretic factor (ANF) receptors in the ciliary processes of the rabbit eye. Exp. Eye Res. 53(5): 591-6.

François, J., 1977. Corticosteroid glaucoma. Ann. Ophthalmol. 9(9): 1075-80.

Fujishiro, T., Kawasaki, H., Aihara, M., Saeki, T., Ymagishi, R., Atarashi, T., Mayama, C., Araie, M., 2014. Establishment of an experimental ferret ocular hypertension model for the analysis of central visual pathway damage. Sci Rep. 4: 6501.

Gaasterland, D., Kupfer, C., 1974. Experimental glaucoma in the rhesus monkey. Invest. Ophthalmol. 13(6): 455-7.

Gelatt, K.N., 1977. Animal models for glaucoma. Invest. Ophthalmol. Vis. Sci. 16(7): 592-6.

Gerometta, R., Podos, S.M., Candia, O.A., Wu, B., Malgor, L.A., Mittag, T., Danias, J., 2004. Steroid-induced ocular hypertension in normal cattle. Arch. Ophthalmol. 122(10): 1492-7.

Gerometta, R., Podos, S.M., Danias, J., Candia, O.A., 2009. Steroid-induced ocular hypertension in normal sheep. Invest. Ophthalmol. Vis. Sci. 50(2): 669-73.

Glovinsky, Y., Quigley, H.A., Dunkelberger, G.R., 1991. Retinal ganglion cell loss is size dependent in experimental glaucoma. Invest. Ophthalmol. Vis. Sci. 32(3): 484-91.

Glovinsky, Y., Quigley, H.A., Pease, M.E., 1993. Foveal ganglion cell loss is size dependent in experimental glaucoma. Invest. Ophthalmol. Vis. Sci. 34(2): 395-400.

Goel, M., Picciani, R.G., Lee, R.K., Bhattacharya, S.K., 2010. Aqueous humor dynamics: a review. Open Ophthalmol. J. 4:52-9.

Gordon, D.M., McLean, J.M., Koteen, H., Bousquet, F.P., McCusker, W.D., Baras, I., Wetzig, P., Norton, E.W., 1951. The use of ACTH and cortisone in ophthalmology. Am. J. Ophthalmol. 34(12): 1675-86.

Grehn, F., Grüsser, O.J., Stange, D., 1984. Effect of short-term intraocular pressure increase on cat retinal ganglion cell activity. Behav. Brain Res. 14(2): 109-21.

Gross, R.L., Ji, J., Chang, P., Pennesi, M.E., Yang, Z., Zhang, J., Wu, S.M., 2003. A mouse model of elevated intraocular pressure: retina and optic nerve findings. Trans. Am. Ophthalmol. Soc. 101: 163-9; discussion 169-71.

Harooni, M., Freilich, J.M., Abelson, M., Refojo, M., 1998. Efficacy of hyaluronidase in reducing increases in intraocular pressure related to the use of viscoelastic substances. Arch. Ophthalmol. 116(9): 1218-21.

Huggert, A., 1957. Obstruction of the outflow of aqueous humour, produced experimentally. Acta. Ophthalmol. (Copenh). 35(1): 1-11.

Inomata, H., Bill, A., Smelser, G.K., 1972. Aqueous humor pathways through the trabecular meshwork and into Schlemm's canal in the cynomolgus monkey (Macaca irus). An electron microscopic study. Am. J. Ophthalmol. 73(5):760-89.

Johnson, M., Erickson, K., 2000. Mechanisms and routes of aqueous humor drainage, in: Albert, D.M., Jakobiec, F.A. (Eds.), Principles and Practice of Ophthalmology. WB Saunders, Philadelphia.

Kalvin, N.H., Hamasaki, D.I., Gass, J.D., 1966. Experimental glaucoma in monkeys. I. Relationship between intraocular pressure and cupping of the optic disc and cavernous atrophy of the optic nerve. Arch. Ophthalmol. 76(1): 82-93.

Kerrigan-Baumrind, L.A., Quigley, H.A., Pease, M.E., Kerrigan, D.F., Mitchell, R.S., 2000. Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons. Invest. Ophthalmol. Vis. Sci. 41(3): 741–748.

Knepper, P.A., Breen, M., Weinstein, H.G., Blacik, J.L., 1978. Intraocular pressure and glycosaminoglycan distribution in the rabbit eye: effect of age and dexamethasone. Exp. Eye Res. 27(5): 567-75.

Knepper, P.A., Goossens, W., Palmberg, P.F., 1996. Glycosaminoglycan stratification of the juxtacanalicular tissue in normal and primary open-angle glaucoma. Invest. Ophthalmol. Vis. Sci. 37(12): 2414-25.

Lanza, R., 2014. Principles of Tissue Engineering, fourth ed. Edited by Robert Lanza, Robert Langer, and Joseph Vacanti. Academic Press.

Lessell, S., Kuwabara, T., 1969. Experimental alpha-chymotrypsin glaucoma. Arch. Ophthalmol. 81(6): 853-64.

Levkovitch-Verbin, H., Quigley, H.A., Martin, K.R., Valenta, D., Baumrind, L.A., Pease, M.E., 2002. Translimbal laser photocoagulation to the trabecular meshwork as a model of glaucoma in rats. Invest. Ophthalmol. Vis. Sci. 43(2): 402-10.

Liesegang, T.J., Bourne, W.M., Ilstrup, D.M., 1986. The use of hydroxypropyl methylcellulose in extracapsular cataract extraction with intraocular lens implantation. Am. J. Ophthalmol. 102(6):723-6.

Mabuchi, F., Aihara, M., Mackey, M.R., Lindsey, J.D., Weinreb, R.N., 2003. Optic nerve damage in experimental mouse ocular hypertension. Invest. Ophthalmol. Vis. Sci. 44(10): 4321-30.

Melena, J., Santafé, J., Segarra-Doménech, J., Puras, G., 1999. Aqueous humor dynamics in alpha-chymotrypsin-induced ocular hypertensive rabbits. J. Ocul. Pharmacol. Ther. 15(1): 19-27.

Morrison, J.C., Fraunfelder, F.W., Milne, S.T., Moore, C.G., 1995. Limbal microvasculature of the rat eye. Invest. Ophthalmol. Vis. Sci. 36(3): 751-6.

Morrison, J.C., Johnson, E., Cepurna, W.O., 2008. Rat models for glaucoma research. Prog. Brain Res. 173: 285-301.

Morrison, J.C., Moore, C.G., Deppmeier, L.M., Gold, B.G., Meshul, C.K., Johnson, E.C., 1997. A rat model of chronic pressure-induced optic nerve damage. Exp. Eye Res. 64(1): 85-96.

Naskar, R., Wissing, M., Thanos, S., 2002. Detection of early neuron degeneration and accompanying microglial responses in the retina of a rat model of glaucoma. Invest. Ophthalmol. Vis. Sci. 43(9): 2962-8.

Paulavičiūtė-Baikštienė, D., Baršauskaitė, R., Janulevičienė, I., 2013. New insights into pathophysiological mechanisms regulating conventional aqueous humor outflow. Medicina (Kaunas). 49(4): 165-9. Review.

Percicot, C.L., Schnell, C.R., Debon, C., Hariton, C., 1996. Continuous intraocular pressure measurement by telemetry in alpha-chymotrypsin-induced glaucoma model in the rabbit: effects of timolol, dorzolamide, and epinephrine. J. Pharmacol. Toxicol. Methods. 36(4): 223-8.

Quigley, H.A., Addicks, E.M., 1980. Chronic experimental glaucoma in primates. I. Production of elevated intraocular pressure by anterior chamber injection of autologous ghost red blood cells. Invest. Ophthalmol. Vis. Sci. 19(2):126-36.

Quigley, H.A., Hohman, R.M., 1983. Laser energy levels for trabecular meshwork damage in the primate eye. Invest. Ophthalmol. Vis. Sci. 24(9): 1305-7.

Roberts, M.D., Liang, Y., Sigal, I.A., Grimm, J., Reynaud, J., Bellezza, A., Burgoyne, C.F., Downs, J.C., 2010. Correlation between local stress and strain and lamina cribrosa connective tissue volume fraction in normal monkey eyes. Invest. Ophthalmol. Vis. Sci. 51(1): 295-307.

Ruiz-Ederra, J., García, M., Hernández, M., Urcola, H., Hernández-Barbáchano, E., Araiz, J., Vecino, E., 2005. The pig eye as a novel model of glaucoma. Exp. Eye Res. 81(5): 561-9.

Ruiz-Ederra, J., Verkman, A.S., 2006. Mouse model of sustained elevation in intraocular pressure produced by episcleral vein occlusion. Exp. Eye Res. 82(5): 879-84.

Samsel, P.A, Kisiswa, L., Erichsen, J.T., Cross, S.D., Morgan, J.E., 2011. A novel method for the induction of experimental glaucoma using magnetic microspheres. Invest. Ophthalmol. Vis. Sci. 52(3): 1671-5.

Sappington, R.M., Carlson, B.J., Crish, S.D., Calkins, D.J., 2010. The microbead occlusion model: a paradigm for induced ocular hypertension in rats and mice. Invest. Ophthalmol. Vis. Sci. 51(1): 207-16.

Sawaguchi, K., Nakamura, Y., Nakamura, Y., Sakai, H., Sawaguchi, S., 2005. Myocilin gene expression in the trabecular meshwork of rats in a steroid-induced ocular hypertension model. Ophthalmic. Res. 37(5): 235-42.

Schubert, H.D., Denlinger, J.L., Balazs, E.A., 1984. Exogenous Na-hyaluronate in the anterior chamber of the owl monkey and its effect on the intraocular pressure. Exp. Eye Res. 39(2):137-52.

Sears, D., Sears, M., 1974. Blood-aqueous barrier and alpha-chymotrypsin glaucoma in rabbits. Am. J. Ophthalmol. 77(3): 378-83.

Sellés-Navarro, I., Villegas-Pérez, M.P., Salvador-Silva, M., Ruiz-Gómez, J.M., Vidal-Sanz, M., 1996. Retinal ganglion cell death after different transient periods of pressure-induced ischemia and survival intervals. A quantitative in vivo study. Invest. Ophthalmol. Vis. Sci. 37(10): 2002-14.

Shareef, S.R., Garcia-Valenzuela, E., Salierno, A., Walsh, J., Sharma, S.C., 1995. Chronic ocular hypertension following episcleral venous occlusion in rats. Exp. Eye Res. 61(3): 379-82.

Sharma S.C., 2003. Intraocular Pressure Elevation: Vein Cauterization, in: Levin, L.A., Di Polo, A. Ocular neuroprotection. Marcel Dekker, New York. Pp: 23-28

Törngren, L., Lundgren, B., Madsen, K., 2000. Intraocular pressure development in the rabbit eye after aqueous exchange with ophthalmic viscosurgical devices. J. Cataract. Refract. Surg. 26(8): 1247-52.

Ueda, J., Sawaguchi, S., Hanyu, T., Yaoeda, K., Fukuchi, T., Abe, H., Ozawa, H., 1998. Experimental glaucoma model in the rat induced by laser trabecular photocoagulation after an intracameral injection of India ink. Jpn. J. Ophthalmol. 42(5): 337-44.

Vareilles, P., Durand, G., Siou, G., Le Douarec, J.C., 1979. Experimental alpha-chymotrypsin model of glaucoma in the rabbit: histopathological studies (author's transl). J. Fr. Ophtalmol. 2(10): 561-8.

Vecino, E., Sharma, S.C., 2011. Glaucoma Animal Models, Glaucoma - Basic and Clinical Concepts, Dr Shimon Rumelt (Ed.), ISBN: 978-953-307-591-4, InTech, Available from: http://www.intechopen.com/books/glaucoma-basic-and-clinical-concepts/glaucoma-animal-models

Wang, L., Cull, G.A., Piper, C., Burgoyne, C.F., Fortune, B., 2012. Anterior and posterior optic nerve head blood flow in nonhuman primate experimental glaucoma model measured by laser speckle imaging technique and microsphere method. Invest. Ophthalmol. Vis. Sci. 53(13): 8303-9.

Weber, A.J., Zelenak, D., 2001. Experimental glaucoma in the primate induced by latex microspheres. J. Neurosci. Methods. 111(1): 39-48.

Wilson, K., McCartney, M.D., Miggans, S.T., Clark, A.F., 1993. Dexamethasone induced ultrastructural changes in cultured human trabecular meshwork cells. Curr. Eye Res. 12: 783–793.

WoldeMussie, E., Ruiz, G., Wijono, M., Wheeler, L.A., 2001. Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. Invest. Ophthalmol. Vis. Sci. 42(12): 2849-55.

Yu, S., Tanabe, T., Yoshimura, N., 2006. A rat model of glaucoma induced by episcleral vein ligation. Exp. Eye Res. 83(4): 758-70.

Zhan, G.L., Miranda, O.C., Bito, L.Z., 1992. Steroid glaucoma: corticosteroid-induced ocular hypertension in cats. Exp. Eye Res. 54(2): 211-8.

Zhu, M.D., Cai, F.Y., 1992. Development of experimental chronic intraocular hypertension in the rabbit. Aust. N. Z. J. Ophthalmol. 20(3): 225-34.

A elaboração desta tese contou com o apoio fundamental de várias pessoas.

Em primeiro lugar, gostaria de agradecer ao Prof. Doutor Amândio Rocha-Sousa, pela pronta disponibilidade com que orientou a elaboração deste trabalho e pela grande oportunidade que me proporcionou de integrar o grupo de Fisiologia da Faculdade de Medicina da Universidade do Porto.

Gostaria também de mandar um grande abraço ao Paulo por toda a ajuda que me foi sempre gratamente prestada, e de agradecer à Ana pela forma como acompanhou e completou o desenvolvimento deste trabalho. Também à Sara, às Ritas e ao Diogo fica o meu mais sincero obrigado por um percurso cheio de bons momentos e com muitas gargalhadas pelo meio.

Quero igualmente agradecer aos meus pais, aos meus avós, à minha irmã e ao André. O apoio da família é sempre importante e foi algo que contribuiu para me dar a força e a confiança necessárias para encarar os meus desafios e alcançar os meus objectivos.

À Rute, a tua dedicação e o teu amor mostraram-me que os sonhos sempre se alcançam quando a pessoa mais especial nos apoia. Encorajaste-me a vencer todas as dificuldades, ficaste sempre ao meu lado quando mais necessitei, e a tua simpatia transformou esta árdua travessia académica numa viagem para nunca mais esquecer.

Anexos

EXPERIMENTAL EYE RESEARCH

The official journal of the International Society for Eye Research

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0014-4835

DESCRIPTION

The primary goal of *Experimental Eye Research* is to publish original research papers on all aspects of **experimental biology** of the **eye** and **ocular tissues** that seek to define the mechanisms of normal function and/or disease. Studies of ocular tissues that encompass the disciplines of **cell biology**, **developmental biology**, **genetics**, **molecular biology**, **physiology**, **biochemistry**, **biophysics**, **immunology** or **microbiology** are most welcomed. Manuscripts that are purely clinical or in a surgical area of ophthalmology are not appropriate for submission to Experimental Eye Research and if received will be returned without review.

Most manuscripts published are original articles describing new research findings. For review purposes the journal is divided into four sections: **Aqueous Humor** and **Blood Flow**; **Cornea** and **Ocular Surface**; **Lens**; and **Retina** and **Choroid**, each with their own section editors and a roster of Executive Editors that have expertise in these specialized areas.

The Journal also publishes review articles, short communications, letters-to-the-editor, and methods papers. Full descriptions of each of these types of articles are detailed in the Guide for Authors.

Research areas include:
Production and circulation of ocular fluids and the dysfunction of these pathways underlying ocular disease
Angiogenesis, neovascularization and regulation of blood flow in the eye in health and disease
Cell biology, molecular biology, biochemistry, and biophysics of the eye or eye tissue
Developmental and regenerative biology of the eye
Human and molecular genetics studies of inherited eye diseases
Gene therapy and neuroprotection targeted at preventing inherited ocular diseases
Neural and general physiology of the visual process

IMPACT FACTOR

2013: 3.017 © Thomson Reuters Journal Citation Reports 2014

ABSTRACTING AND INDEXING

BIOSIS
Chemical Abstracts
Current Contents/Life Sciences
MEDLINE®
EMBASE
Research Alert
Excerpta Medica
Scopus

EDITORIAL BOARD

GUIDE FOR AUTHORS

INTRODUCTION

The goal of *Experimental Eye Research* is to publish original research papers on all aspects of the cell biology, physiology, genetics, biochemistry, biophysics, molecular biology, biophysics, pharmacology, developmental biology, microbiology, and immunology of the eye. The journal is subdivided into four sections; Aqueous Humor and Blood Flow, Cornea and Ocular Surface, Lens and Retina and Choroid, each with their own section editors. Short Communications, Letters to the Editor, Methods in Eye Research; individual Review Articles or collections of Review Articles specifically commissioned by the Journal are also published.

Research areas include:
Production and circulation of ocular fluids and the dysfunction of these pathways underlying ocular disease Angiogenesis, neovascularization and regulation of blood flow in the eye in health and disease Cell biology, molecular biology, biochemistry, and biophysics of the eye or eye tissueDevelopmental and regenerative biology of the eyeHuman and molecular genetics studies of inherited eye diseasesGene therapy and neuroprotection targeted at preventing inherited ocular diseasesNeural and general physiology of the visual process

Types of communications

1. **Research Articles** :Original Research Articles describing the results of experimental studies that address fundamental biological issues on vision, the eye, or specific ocular tissues constitutes the majority of communications published in *Experimental Eye Research*. Detailed instructions for formatting regular research articles are provided below under the subheading **"Preparation"**.

2. **Letters to the Editor:**Letters to the Editor should provide substantive comment(s) on a publication in this Journal or an eye research article published elsewhere; or on issues of broad interest to the eye and vision research community. A Letter should be concise, to the point (generally no more than 750 words), contain only text (no abstract, figures, tables, acknowledgments, or reference list), and be written in continuous narrative style (no headings/subheadings). The Editor-in-Chief or a designated member of the Editorial Board will be responsible for reviewing Letters. Receipt of a Letter does not guarantee that it will be accepted for publication. In the event that the Letter challenges some aspect of a prior publication, a complete citation of the publication in question should be fully spelled out in the body of the text. The authors of the publication in question will be given the opportunity to respond to the comments made, and the two Letters (if accepted) will be published sequentially in the same issue of the Journal.

3. **Short Communications:** Short Communications are intended for preliminary reports of original, significant research results that are limited in scope and, thus, do not warrant publication in the form of a regular Research Article. Communications should be no longer than 4,500 words (generally not to exceed 4 printed pages in the Journal), inclusive of all literature citations, and should contain no more than two Figures (which may be multi-panel) and/or Tables;"Supplementary Data" is not permitted. The word count pertains only to the main body of text, excluding the title, author/institution details, abstract, figures/tables, figure legends, and acknowledgments; the Abstract should not exceed 250 words. Communications should not contain headings/subheadings (*i.e.,*Introduction, Materials and Methods, Results, Discussion), other than References, but otherwise should follow the rules pertaining to the preparation, text-formatting and submission of Research Articles for this Journal.

4. **Focus on Molecules:**Focus on Molecules articles are no longer accepted by Experimental Eye Research.

5. **Methods in Eye Research:**These feature articles provide a detailed overview of a specific method or technique used in experimental research of the visual system. This contribution should contain sufficient information to allow successful reproduction of the experimental method/technique in another laboratory. Each article should contain the following headings (the first four being numbered):IntroductionMaterials and SuppliesDetailed MethodsPotential Pitfalls and Trouble ShootingReferences*Article Specification:*
The article should not exceed 12 published pages in length including equivalent space for figures. For members of ISER, colour figures will be printed without charge.
Include enough detailed information to allow researchers in independent laboratories to successfully reproduce this method

Please highlight the potential "problem areas" for the method and provide "trouble shooting" solutions Please ensure you select the correct article type (Methods in Eye Research) when uploading your article via http://ees.elsevier.com/yexer. If you would like to submit an unsolicited Methods In Eye Research article for consideration, or if you have any editorial queries, please , please contact the Methods in Eye Research Editor, Dr. Abe Clark, at abe.clark@unthsc.edu.

6. **Special Issues:** Periodically, Experimental Eye Research will publish a special issue containing review articles that cover selected topics in depth relevant to eye research. While the breadth and scope of these special issues can vary widely, they are intended to contain in a single issue the state of the art in specific areas of eye research. Up to four color plates will be published free of charge in each review articles commissioned by the journal. If you are interested in developing a special issue, please contact the Editor-in-Chief, or the *Special Issues and Review Articles Editor,* Dr. Steven J. Fliesler, at: fliesler@buffalo.edu. Each review included in a special issue will undergo peer review before being accepted for publication.

7. **Review Articles:** Single review articles are periodically published in Experimental Eye Research. Most published review articles are solicited, but the Editor-in-Chief is always willing to consider new topics for a review. Prior to preparing a review article it is important to first contact the Editor-in-Chief, or Dr. Steven J. Fliesler, *Special Issues and Review Articles Editor,* (fliesler@buffalo.edu) as to whether such a review would be appropriate for publication consideration. No reviews will be published without full peer review. We want all reviews to be succinct and pithy. While the length of a review will be governed by the scope of the topic covered, we suggest to authors that the length be approximately 6000 words, including space for tables, figures and references. Up to four color plates will be published free of charge in each review articles commissioned by the journal.

Contact details for submission

Experimental Eye Research Editorial Office, 525 B Street, Suite 1800, San Diego, CA 92101-4495, USA Tel.: (619) 699-6278; Fax: (619) 699-6850; E-mail: exer@elsevier.com

BEFORE YOU BEGIN

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Human and animal rights

If the work involves the use of animal or human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans http://www.wma.net/en/30publications/10policies/b3/index.html; EU Directive 2010/63/EU for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; Uniform Requirements for manuscripts submitted to Biomedical journals http://www.icmje.org. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsofinterest. Further information and an example of a Conflict of Interest form can be found at: http://help.elsevier.com/app/answers/detail/a_id/286/p/7923.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/sharingolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck http://www.elsevier.com/editors/plagdetect.

Contributors
Each author is required to declare his or her individual contribution to the article: all authors must have materially participated in the research and/or article preparation, so roles for all authors should be described. The statement that all authors have approved the final article should be true and included in the disclosure.

Addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts
Before the accepted manuscript is published in an online issue Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include:The reason the name should be added or removed or the author names rearranged. Written confirmation (email, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: Journal Managers will inform the Journal Editors of any such requests. Publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.

After the accepted manuscript is published in an online issue
Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:
Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.
After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see http://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

For open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see http://www.elsevier.com/OAauthoragreement). Permitted third party reuse of open access articles is determined by the author's choice of user license (see http://www.elsevier.com/openaccesslicenses).

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. For more information see http://www.elsevier.com/copyright.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some authors may also be reimbursed for associated publication fees. To learn more about existing agreements please visit http://www.elsevier.com/fundingbodies.

Open access

This journal offers authors a choice in publishing their research:

Open access

• Articles are freely available to both subscribers and the wider public with permitted reuse

• An open access publication fee is payable by authors or on their behalf e.g. by their research funder or institution

Subscription

• Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs (http://www.elsevier.com/access).

• No open access publication fee payable by authors.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)

Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The open access publication fee for this journal is **USD 2200**, excluding taxes. Learn more about Elsevier's pricing policy: http://www.elsevier.com/openaccesspricing.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Submit your article
Please submit your article via http://ees.elsevier.com/yexer.

Referees
Please submit, with the manuscript, the names, addresses and e-mail addresses of 5 potential referees. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

US National Institutes of Health (NIH) voluntary posting (" Public Access") policy Elsevier facilitates author posting in connection with the voluntary posting request of the NIH (referred to as the NIH "Public Access Policy", see http://www.nih.gov/about/publicaccess/index.htm) by posting the peer-reviewed author's manuscript directly to PubMed Central on request from the author, after formal publication. Upon notification from Elsevier of acceptance, we will ask you to confirm via e-mail (by e-mailing us at NIHauthorrequest@elsevier.com) that your work has received NIH funding (with the NIH award number, as well as the name and e-mail address of the Prime Investigator) and that you intend to respond to the NIH request. Upon such confirmation, Elsevier will submit to PubMed Central on your behalf a version of your manuscript that will include peer-review comments, for posting 12 months after the formal publication date. This will ensure that you will have responded fully to the NIH request policy. There will be no need for you to post your manuscript directly to PubMed Central, and any such posting is prohibited. Individual modifications to this general policy may apply to some Elsevier journals and its society publishing partners.

PREPARATION

Use of word processing software
It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

LaTeX
You are recommended to use the Elsevier article class *elsarticle.cls* (http://www.ctan.org/tex-archive/macros/latex/contrib/elsarticle) to prepare your manuscript and BibTeX (http://www.bibtex.org) to generate your bibliography.
For detailed submission instructions, templates and other information on LaTeX, see http://www.elsevier.com/latex.

Article structure
Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract of no more than 500 words is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. The abstract should be in paragraph form with no abbreviations or subheadings.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images and in accordance with all technical requirements: Illustration Service.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See http://www.elsevier.com/highlights for examples.

Keywords

Immediately after the abstract, provide a maximum of 8 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations
Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units
Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI. You are urged to consult IUPAC: Nomenclature of Organic Chemistry: http://www.iupac.org/ for further information.

Database linking
Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

Accession numbers
Accession numbers are unique identifiers in bioinformatics allocated to nucleotide and protein sequences to allow tracking of different versions of that sequence record and the associated sequence in a data repository [e.g., databases at the National Center for Biotechnical Information (NCBI) at the National Library of Medicine ('GenBank') and the Worldwide Protein Data Bank]. There are different types of accession numbers in use based on the type of sequence cited, each of which uses a different coding. Authors should explicitly mention the *type of accession number together with the actual number*, bearing in mind that an error in a letter or number can result in a dead link in the online version of the article. Please use the following format: accession number type ID: xxxx (e.g., MMDB ID: 12345; PDB ID: 1TUP). Note that in the final version of the *electronic copy*, accession numbers will be linked to the appropriate database, enabling readers to go directly to that source from the article.

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork
Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF, EPS or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color in print or on the Web only.

There is no charge for colour in print for members of ISER, or for *invited* Reviews.

For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.

Please note: Because of technical complications which can arise by converting color figures to "gray scale" (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations: http://www.issn.org/services/online-services/access-to-the-ltwa/.

Video data
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

AudioSlides
The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and

to help readers understand what the paper is about. More information and examples are available at http://www.elsevier.com/audioslides. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Supplementary material

Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

3D radiological data

You can enrich your online article by providing 3D radiological data in DICOM format. Radiological data will be visualized for readers using the interactive viewer embedded within your article, and will enable them to: browse through available radiological datasets; explore radiological data as 2D series, 2D orthogonal MPR, 3D volume rendering and 3D MIP; zoom, rotate and pan 3D reconstructions; cut through the volume; change opacity and threshold level; and download the data. Multiple datasets can be submitted. Each dataset will have to be zipped and uploaded to the online submission system via the '3D radiological data' submission category. The recommended size of a single uncompressed dataset is 200 MB or less. Please provide a short informative description for each dataset by filling in the 'Description' field when uploading each ZIP file. Note: all datasets will be available for download from the online article on ScienceDirect. So please ensure that all DICOM files are **anonymized** prior to submission. For more information see: http://www.elsevier.com/about/content-innovation/radiological-data

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

- E-mail address
- Full postal address

All necessary files have been uploaded, and contain:

- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations

- Manuscript has been 'spell-checked' and 'grammar-checked'
- References are in the correct format for this journal
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)

Printed version of figures (if applicable) in color or black-and-white

- Indicate clearly whether or not color or black-and-white in print is required.
- For reproduction in black-and-white, please supply black-and-white versions of the figures for printing purposes.

For any further information please visit our customer support site at http://support.elsevier.com.

AFTER ACCEPTANCE

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. Example of a correctly given DOI (in URL format; here an article in the journal *Physics Letters B*):
http://dx.doi.org/10.1016/j.physletb.2010.09.059

When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author, at no cost, will be provided with a personalized link providing 50 days free access to the final published version of the article on ScienceDirect. This link can also be used for sharing via email and social networks. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's WebShop (http://webshop.elsevier.com/myarticleservices/offprints). Authors requiring printed copies of multiple articles may use Elsevier WebShop's 'Create Your Own Book' service to collate multiple articles within a single cover (http://webshop.elsevier.com/myarticleservices/booklets).

AUTHOR INQUIRIES

You can track your submitted article at http://help.elsevier.com/app/answers/detail/a_id/89/p/8045/. You can track your accepted article at http://www.elsevier.com/trackarticle. You are also welcome to contact Customer Support via http://support.elsevier.com.

Apêndice

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

Serviço de Fisiologia

Porto, March 23rd 2015

Dear Editor,

We are pleased to submit the article manuscript entitled "Experimental animal models of induced intraocular hypertension" by *Coimbra M., Moleiro A., Rocha-Sousa A.* to **Experimental Eye Research Journal** for appreciation.

Sincerely yours,

Miguel Trigo Coimbra

Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

Tel: 351 22 5513644; Fax: 351 22 5513646; e.mail: amoreira@med.up.pt