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Assinatura conforme cartão de identificação:

Miguel Trigo Coimbra

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

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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 etiologic 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- β 2, 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 chymotrypsin 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 μ m 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 μ m. This is analogous to the modern intraocular microsphere obstruction mechanism, which is described as occurring preferentially at the interlamellar 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 2×10^5 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 3×10^6 beads per μL), 2 μL of 1 μm beads (at 1.5×10^7 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](#)). [Törnngren 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 adrenocorticotrophic hormone ([Gordon et al., 1951](#)). Later, [François \(1977\)](#) described how steroids inhibit the lysosomal 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 excavation 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 ocuopression	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. ^aPre-trabecular refers to models which simulate mostly an unbalanced increase in aqueous humor inflow. ^bTrabecular refers to models which induce obstruction or destruction of the trabecular meshwork. ^cPost-trabecular indicates models which preferentially decrease aqueous humor outflow through the “conventional” pathway downstream from the trabecular meshwork, causing vein congestion.

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Anexos



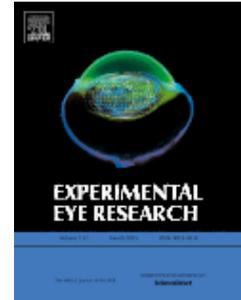
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Apêndice

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,

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