A COMPARISON OF TWO EXPERIMENTAL RABBIT MODELS OF GLAUCOMA

L. Ignacio Tártara[1,2,4], Stefania Leavi[1], Vilma R. Campana[1,3], Daniel A. Allemandi[2], Santiago D. Palma[2].

Abstract:

Objective: to compare two experimental models of glaucoma by inducing ocular hypertension in rabbits and describing the anatomopathological changes observed.
Methods: We used 16 female New Zealand rabbits, weighing 2-3 kg.
Model A (n=6): Cauterization of the episcleral and perilimbar veins of the right eye (RE) using surgical electrocautery.
Model B (n = 10): Intracamerular injection of α-chymotrypsin into the RE.
We measured the intraocular pressure (IOP) before and after inducing intraocular hypertension once per week at the same time of the day, during 40 days, with a manual tonometer. The animals were sacrificed by CO2 inhalation. For both models, IOP in the left eye (LE) was selected as the control value. The mean and standard error (SE) of the IOP values, expressed in mmHg, were evaluated and compared statistically applying Student's T-test, considering $p < 0.05$ a significant value.
Results: IOP in the LE was 12,9±1,05 in model A and 12,9±1,09 in model B. No significant differences were observed.
Model A: there was a 14,7% increase in the RE IOP (14,8±1,4) with respect to the LE IOP. A significant increase in IOP was observed within the first 25 hours 23,5±1,9 ($p<0,05$), as compared with the IOP value of the control eye. There were no significant differences in subsequent measurements.
Model B: the increase in IOP was of 129,1% (29,6±3,4) as compared to the LE. In all cases an increase in IOP was observed from Day 1 ($p<0.05$). The RE IOP peaked on Day 25: 35±3.4 ($p<0.05$).
The increase in IOP induced by model B was significantly higher ($p<0.01$) than the increase obtained with model A. Retinal ganglion cell loss was observed in both models, but buphthalmos, subluxation of the lens and increased excavation of the papilla were observed only in model B.
Conclusion: This study indicates that model B is the most appropriate method for inducing a rapid, controlled increase of IOP in rabbits and, more importantly, that this increase can be sustained over extended periods of time. This model could be useful for evaluating the efficacy of new ocular drug delivery systems and for further studies of the physiopathology of glaucoma.

Key words: Experimental glaucoma model. A-chymotrypsin. Episcleral vein cauterization. Ocular hypertension.

1 Cátedra de Física Biomédica. Facultad de Ciencias Médicas. UNC
2 Departamento de Farmacia. Facultad de Ciencias Químicas. UNC
3 Cátedra de Física Biomédica. Medicina. UNLaR
4 Email de contacto: i.tartara@gmail.com

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Introduction

The term "glaucoma" describes a group of eye disorders with multifactorial etiology, all of which are characterized by progressive optic neuropathy [1]. It is a disorder that compromises retinal ganglion cells (RGC), leading to their degeneration and death, visual field loss and, eventually, blindness. It is one of the main causes of blindness and visual impairment affecting the world population, and it is estimated that there are 70 million people that suffer from this disease [2, 3].
The most important risk factor associated with glaucoma is increased intraocular pressure (IOP), and it is the only one that is amenable to intervention [4]. The death of retinal neurons is the bridge between glaucoma and retinal disease [5]. As such, hypotensive and neuroprotective therapies are an imperative therapeutical need, which is essential for patients suffering from glaucoma [6].
In spite of the efforts and research on the field of glaucoma, its physiopathology has not yet been entirely clarified. Animal models have greatly improved our understanding of the causes of human diseases and their progression, and have shown to be a useful tool in discovering targets for pharmacological and surgical interventions. Even so, several conditions remain incurable because not all of the models used to study them can be completely reproduced [7].
In the study of galucoma both large (monkeys [8], dogs [9], cats [10] and pigs [11]) and small (rats, guinea pigs, and rabbits [12]) animals have been used. Several different methods for inducing experimental glaucoma have been implemented, like the use of laser, episcleral vein obstruction, the injection of different substances at the intraocular or systemic level, the application of general and topical corticosteroids, etc [13].
As it was previously mentioned, experimental models are essential for studying the natural course of glaucoma and for developing therapeutic interventions that can be of use in stopping or reverting the progression of the disease [14]. Using mammals like rabbits enables us to reproduce excellent models, based on their anatomical similarities with humans, with which surgical procedures similar to those of clinical ophthalmology can be used.
Considering all that we mentioned above, we set out to reproduce and compare to models of glaucoma by ocular hypertension induction in rabbits, describing the pathological anatomical changes observed.

Methods

We used 16 female albino New Zealand rabbits, 2-3 kg. The rabbits received food and water ad libitum in a room at controlled temperature (21°±5°C), and were exposed to 12-hours-long cycles of darkness and light.
All experimental procedures met the ARVO (Association for Research in Vision and Ophthalmology) norms and the CCED on the protection of animals used for scientific purposes (86/609/CEE). All protocols were revised and approved by the *Comité de Uso y Cuidado Institucional de Animales de la Facltad de Ciencias Químicas de la Universidad Nacional de Córdoba,* Córdoba, Argentina, 342/2009 resolution emitted by the HCD of the FCQ. After a one week long adaptation period, the animals were admitted to the experimental sessions.
Model A (n = 6): cauterization of the episcleral veins [15] and perilimbal vessels of the right eye by electrocautery.
Surgical procedure: we carried out a 360-degree conjunctival peritomy, located three episcleral veins, and cauterized them with a fine tip portable bipolar cautery pen (HTCF726600). Then, we caused 15 perilimbal burns with the same cautery, and closed the conjunctiva with 9.0 nylon. We applied antibiotics and topical non-steroidal antinflammatory drugs during 7 days.
Model B (n = 10): intracamerular injection of α-chymotrypsin in the right eye [16].
Surgical procedure: We instilled 2% pilocarpine to induce miosis, after which parecentesis was performed on the cornea and 0.1 ml (3mg/ml) of chymotrypsin (C425-250mg Sigma lab) were injected into the posterior chamber with a 27G cannula. After three minutes the anterior chamber was washed with sterile physiological solution. Antibiotics and topical non-steroidal antiinflammatory drugs were applied during 7 days. 0.1 ml of sterile physiological solution were injected into the posterior chamber of the left eye, which was the eye used as control.
For both models IOP was measured before and after inducing ocular hypertension every five days during 40 days with a properly calibrated manual tonometer (Icare TonoVet. Icare USA). Digital photographs of the anterior segment and fundus were taken with a camera attached to the slit lamp (Huvitz HIS 5000. south Corea). All animals received xylazine (0.2 ml/kg) and ketamine (0.8 ml /kg) for general anesthesia. In both models, the control values were the IOP of the left eye, expressed in mmHg. When the tests were completed, the animals were sacrificed by CO_2 inhalation after being sedated with xylacine (0.2 ml/kg). Both eyes were enucleated *ex vivo*, histological samples were prepared using haematoxylinand and eosin staining, and a descriptive analysis of the retinae was carried out (layer of retinal ganglion cells), comparing the eyes with normal intraocular tension and the eyes with intraocular hypertension through optical microscopy (optical microscope Olympus BX41. Japan).
Statistical analysis: the results were analyzed applying Student's T-test (individual times), and MANOVA (Hotelling's T) to compare the IOP in both models, considering $p<0.05$ to be a significant level.

Results

There were no significant differences in the left eye intraocular pressure between Model A and Model B (Model A LE IOP was 12,9±1,05, and Model B LE IOP was 12.9±1.09)

Model A (see Figure 1): the increase in the IOP of the right eye was 14.7% (14.8±1.4), as compared to the left eye. A significant increase in IOP was observed within the first 24 hours: 23.5±1.9 ($p<0.05$) as compared to the control eye. There were no significant differences in later controls.

Model B (see Figure 1): the increase in IOP was of 129,1% (29.6±3.4), compared to the left eye. In all cases we observed an increase from the first day (p>00.5). The peak of IOP in the right eye was observed at the 25th day: 35±3.4 ($p<0.05$).

The increase in IOP induced by Model B (29.6±3.4) was significantly higher ($p<0.01$) than the increase induced by model A (14.8±1.4).

On model A we observed a moderate to low decrease in RGC counts (Figure 2b) as compared to the normal retinal extracts (Figure 2b), while on model B we observed a marked drop in RGC (figure 2b). Only in model B pathological anatomical changes were observed: buphthalmos (figure 3a and 3b), lens subluxation (figure 3c and 3d), and an increase in papillary excavation (figure 3f).

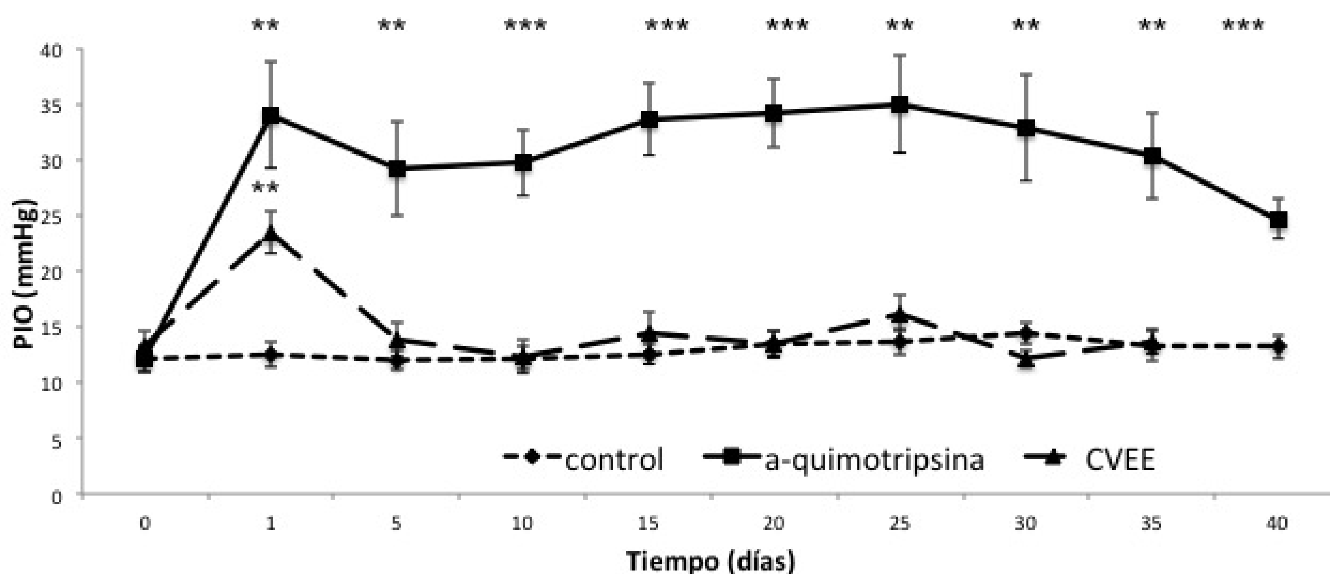

Figure 1: Comparison of intraocular pressure in normal rabbit eyes and intraocular pressure in Model A and B. The dotted line representes the intraocular pressure in the control eyes (n=16), and the discontinuous line represents the intraocular pressure values in the eyes treated following Model A (electrocautery), (n=6). Lastly, the continuous line indicates the value of intraocular pressure in the right eye of the rabbits in Model B (n=10). Each ordered pair represents the mean ± SE intraocular pressure value at each time, expressed in mmHg. **p < 0,01 ***p < 0,001

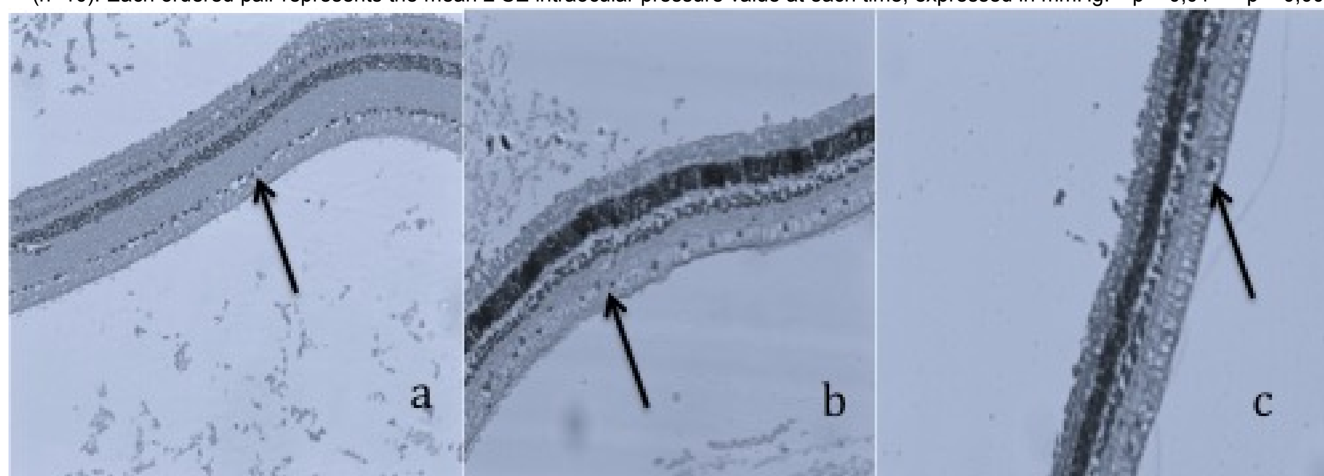

Figure 2: Photographs of samples extracted from the rabbits' retinae. H&E, 10X. Photograph a) Eye with normal intraocular pressure; b) Model A eye; c) Model B eye. The arrows point to the ganglion cell layer.

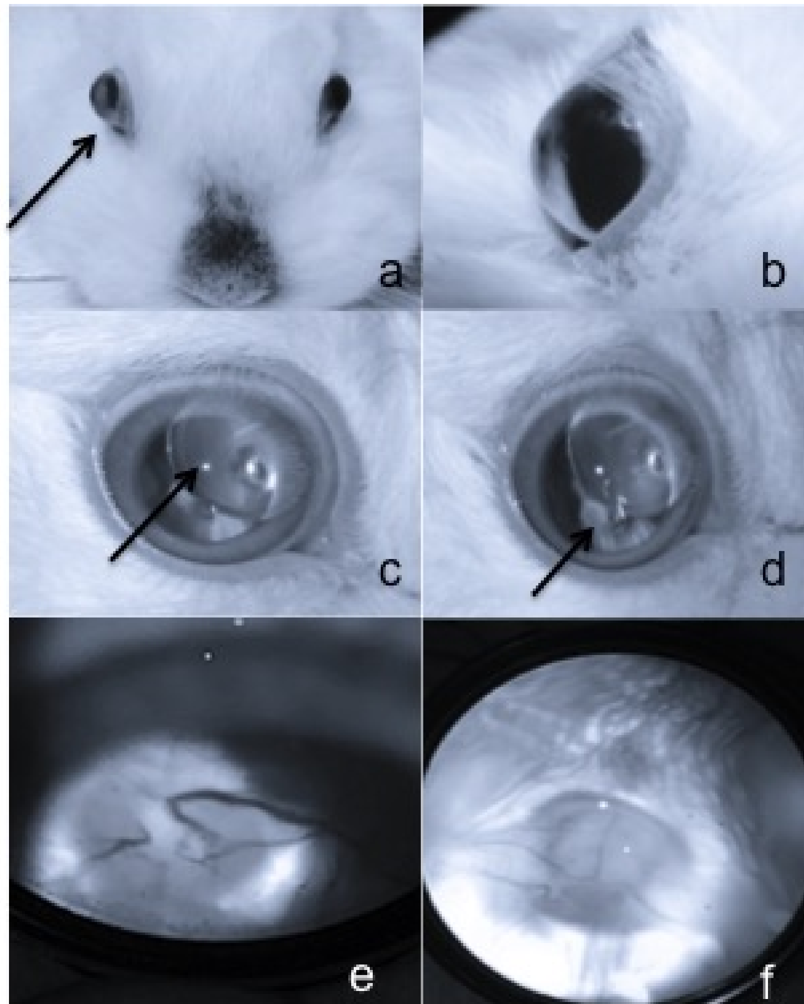

Figure 3: a– Photograph of a rabbit *(model B),* whose right eye is enlarged as a consequence of intraocular hypertension (buphthalmos). Photograph –b– shows in detail the enlargement of the eye globe, the ballooning of the cornea, and the enlargement of the anterior chamber. In photograph –c–, lens subluxation can be observed, with an arrow pointing to the lens, and in photograph –d– the arrow shows retroillumination from the fundus. –e– is a photomicrograph of the optic nerve of the rabbit in its normal state. –f– is a photograph of the optic nerve 20 days after the injection of α-chymotrypsin, with increased papillary excavation (model B).

Discussion

Glaucoma is a complex disease whose physiopathology is far from being fully understood. It is likely that the continuous development of animal models and the in-depth probing of the disease contribute to the future clarification

Glaucoma is a complex disease whose physiopathology is far from being fully understood. It is likely that the continuous development of animal models and their use in exploring the disease in depth contribute to the future clarification of its varied characteristics and its treatment in human patients. Rodent models are particularly useful due to their ready availability, low cost, short lifespan and due to the ease with which they can be experimentally handled and genetically manipulated [17]. However, these models do not reproduce human conditions exactly. The eye of a rabbit is anatomically very similar to a human eye, and rabbits are easy to handle and are relatively cheap. Taking this into consideration, they were selected for their application in experimental glaucoma.

CVEE is a technique that has been used for inducing increases in IOP in rats by cauterizing one or more episcleral veins, blocking the drainage of aqueous humor [18].

In our research we used a modified version of this model, cauterizing the perilimbal vessels of rabbits (Model A). This enabled us to produce a 15% increase in IOP in all rabbits, although it only lasted 24 hours. The results of our experiment differed markedly from the results obtained by Ruiz-Ederra and Verkman [15], who applied this technique on mice and obtained increases in IOP of around 100%, which persisted for 4 weeks. It is likely that the mechanisms governing intraocular pressure in mice and rabbits differ. These mechanisms are described by Morrison *et al.* [19], who suggested that increases in ocular vein congestion slow the rate at which aqueous humour drains through the organizational units of the tubes in Schlemm's canal. Using this model, Garcia-Valenzuela et al. [20] determined that the number of cauterized episcleral veins correlates with the degree to which IOP increases, and determined that ocular

hypertension causes the amount of retinal ganglion cells to drop at a rate of approximately 4% every week. Furthermore, they demonstrated that the death of at least part of the lost retinal ganglion cells occurs due to apoptosis. In our case, only three episclearal veins were cauterized, since our attempts to cauterize more veins caused postsurgical complications like hyphema and corneal edema in all cases, which forced us to exclude the affected rabbits from the study. The descriptive analysis of the samples (fig 4 b) showed that there was retinal ganglion cell loss, but it was not possible to determine the pace at which it occurred or to measure the amount of the decrease, since the period during which intraocular pressure was high was very brief (24 hours).

Another technique for inducing increases in IOP in animal models is the injection of α-chymotrypsin in the posterior chamber [21, 22]. Although currently other procedures are preferred, using this model we obtained results that diferred from those obtained with model A. The increase in IOP was approximately 130%, stable, and peaked at around 35 mmHg three weeks after the intervention. Similar results were published by Best et al. [16], who injected 75 units of α-chymotrypsin in the posterior chamber, causing increases in IOP that rarely were higher than 50 mmHg, and lasted a year or more. Vareilles *et al.* [23] reported findings that were similar to those of our study, like progressive buphthalmos, which appeared during the first two or three weeks after injecting the enzyme, changes in eye cells that included keratopathy, iris atrophy, ciliary body atrophy, and papillary excavation. The retinal nerve fiber layer thinned, and we observed that there was a loss of retinal ganglion cells while other retinal cells were preserved. During the experiments we also observed lens subluxation.

The mechanism that leads to glaucoma after the injection of α-chymotrypsin is not clear. Probably the inflammation caused by the enzyme leads to synechiae like the ones described by Best *et al. [16]*, blocking the drainage of aqueous humor through the trabecular meshwork. Chee et al. [24] determined that trabecular meshwork blockage in Rhesus monkeys was due to lysis in the zonular material. Lessel et al. [25] posit that the ciliary body atrophies.

Although model A enabled us to induce an increase in IOP in rabbits, IOP levels dropped after a short period, and the increase obtained was significantly lower than that obtained using model B. Moreover, the first model requires specialist surgical skills, performing the technique is time-costly, and it has a steep learning curve. As regards the anatomopathological changes observed upon examining the rabbits, buphthalmos (enlargening of the eye caused by intraocular hypertension) (figure 3a and b), lens subluxation due to the effect of the enzyme and increases in eye diameter causing zonular fiber lysis (figure 3c and d), paralytic mydriasis (an indirect sign of blindness), and increases in papillary excavation (which indicates there was a significant loss of retinal nerve cells) were present in all the rabbits treated with the second technique, in addition to significant retinal ganglion cell loss (figure 2 c). None of these changes were found in rabbits treated with model A, except a mild to moderate loss of retinal ganglion cells (figure 2b). These differences were due to the differences in the amount of time during which the animals in each model suffered ocular hypertension.

Conclusions

According to our study, model B appears to be the most appropriate for inducing a fast and controlled increase in intraocular pressure in rabbits and, more importantly, the increase persists over extended periods. This model could be of use in evaluating the efficacy of new ocular systems for drug release and in carrying out future studies on the physiopathology of glaucoma.

Conflict of interest

The authors have no economic interest over the information contained in the manuscript, nor potential conflicts of interest.

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References

1. Casson RJ, Chidlow G, Wood JP, Crowston JG, Goldberg I. Definition of glaucoma: clinical and experimental concepts. Clin Exp Ophthalmol 2012; 40(4): 341–9.
2. Quigley H, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J of Ophthalmol 2006; 90(3): 262–7.
3. Thylefors B, N´egrel AD, Pararajasegaram R, Dadzie KY. Available data on blindness (update 1994). Ophthalmic Epidemiol 1995; 2(1): 5-39.
4. The AGIS Investigators. The advanced glaucoma intervention study (AGIS) VII. The relationship between control of intraocular pressure and visual field deterioration. A J Ophthalmol. 2000; 130(4): 429–40,
5. Garcia-Campos J, Villena A, Diaz F, Vidal L, Moreno M, Perez de Vargas I. Morphological and functional changes in experimental ocular hypertension and role of neuroprotective drugs. Histol Histopathol 2007; 22(12): 1399-1411.

6. Bagnis A, Papadia M, Scotto R, Traverso CE. Current and emerging medical therapies in the treatment of glaucoma. Expert Opin Emerg Drugs 2011; 16(2): 293-307.
7. Vecino E. Animal models in the study of the glaucoma: past, present and future. ArchSocEspOftalmol 2008; 83(9): 517–19.
8. Rasmussen CA, Kaufman PL. Primate glaucoma models. J Glaucoma 2005; 14(4): 311–14.
9. Brooks DE. Glaucoma in the dog and cat. Vet Clin North Am Small Anim Prac 1990; 20(3):775–97.
10. Dietrich U. Feline glaucomas. Clin Tech Small An P 2005; 20(2):108–16.
11. Ruiz-Ederra J, García M, Hernández M, et al. The pig eye as a novel model of glaucoma. Exp Eye Res 2005; 81(5):561–69.
12.Pang IH, Clark AF. Rodent models for glaucoma retinopathy and optic neuropathy. J Glaucoma 2007; 16(5): 483–505.
13. Zeiss CJ. Translational models of ocular disease. Vet Ophthalmol 2013; 16(1): 15-33.
14. Agarwal R, Agarwal P. Rodent models of glaucoma and their applicability for drug discovery. Expert Opin Drug Discov 2017; 12(3): 261-270.
15. Ruiz-Ederra J, Verkman AS. Mouse model of sustained elevation in intraocular pressure produced by episcleral vein occlusion. Exp Eye Res 2006; 82(5): 879–84.
16. Best M, Rabinovitz AZ, Masket S. “Experimental α-chymotrypsin glaucoma”. Ann Ophthalmol. 1975; 7(6): 803-10.
17. Yu S, Tanabe T, Yoshimura N. A rat model of glaucoma induced by episcleral vein ligation. Exp Eye Res 2006; 83(4): 758–70.
18. Shareef SR, Garcia-Valenzuela E, Salierno A, Walsh J, Sharma SC. Chronic ocular hypertension following episcleral venous occlusion in rats. Exp Eye Res 1995; 61(3): 379–82.
19. Morrison JC, Moore CG, Deppmeier LMH, Gold BG, Meshul CK, Johnson EC. A rat model of chronic pressure-induced optic nerve damage. Exp Eye Res 1997; 64(1): 85–96.
20.Garcia-Valenzuela E, Shareef S, Walsh J, Sharma SC. Program med cell death of retinal ganglion cells during experimental glaucoma. Exp Eye Res. 1995; 61(1): 33-44.
21. Zhu MD, Cai FY. Development of experimental chronic intraocular hypertension in the rabbit. Aust N Z J Ophthalmol.1992; 20(3): 225-34.
22. Melena J, Santafe J, Segarra J. The effect of topical dihydroergocristine on the intraocular pressure in alpha-chymotrypsin-induced ocular hypertensive rabbits. Methods Find Exp Clin Pharmacol 1998. 20(10): 861-867.
23. Vareilles P, Durand G, Siou G, Le Douarec JC. Experimental alpha-chymotrypsin model of glaucoma in the rabbit: histopathological studies. J Fr Ophtalmol. 1979; 2(10): 561-8.
24. Chee P, Hamasaki DI. The Basis for Chymotrypsin-Induced Glaucoma. Arch Ophthalmol 1971; 85(1): 103-06.
25. Lessell S, Kuwabara T. Experimental alpha-chymotrypsin glaucoma. Arch Ophthalmol 1969; 81(6): 853–64.