Chapter 2

Experimental Glaucoma After Oxidative Stress and Modulation of the Consequent Apoptotic Events in a Rat Model

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1. Introduction

Glaucoma derives from an increase of the intra-ocular pressure (IOP) due to accumulation of the aqueous humor which causes degenerative events at the level of the retina and the optic nerve. This results in a progressive damage of the optic nerve that is paralleled by the gradual loss of retinal ganglion cells (RGC). The pathology causes increasing eyesight deterioration particularly in the peripheral areas of the visual field. The optic nerve papilla becomes paler and shows an augmented excavation as compared with a normal physiological situation. The increase of the IOP is to be ascribed, in the majority of cases, to an alteration of the ocular hydrodynamics: in particular the normal efflux of aqueous humor from the anterior chamber of the eye is severely hindered. The drainage system is located in the limbal regions or in the sclero-corneal junction. The inner surface presents a hollow (depression) known as inner scleral spur which is filled by the trabecular meshwork and the canal of Schlemm. Primary open angle glaucoma is caused by the failure of drainage from the trabecular meshwork, while the primary closed angle glaucoma consists in a modification of the iris-corneal angle. It is commonly accepted that glaucoma is the second cause of blindness in the world; as a matter of fact it has been estimated that 68 millions of patients are affected by this pathology and out of them, about 7 millions suffer complete bilateral blindness as a consequence of the glaucoma. The onset of the disease may occur at any age, also at childhood, but it is significantly more frequent in elderly people. Glaucoma is generally categorized in five different groups; two of them are the above mentioned open and closed angle primary glaucoma which are also the most widespread ones. A broad variety of pathological conditions may induce, as secondary

event, the obstruction of the drainage system of the drainage angle which results in glaucoma. The primary open angle, which represents more than 60% of the cases, is a chronic condition. The outflow angle is not altered; the aqueous humor produced by the ciliary body reaches the trabecular meshwork, but its drainage is not efficient. This is possibly due to the decrease of diffusion towards the Schlemm's canal which causes a continuing increase of the IOP ending in the progressive degeneration of the optic nerve. Among the secondary factors contributing to the insurgence of glaucoma one should take into account: age (above 70), myopia and ethnic origin since the African populations seem to be more prone to develop the disease.

In the primary closed angle glaucoma which occurs in about 10% of patients, a closure of the filtration angle in the eye is observed and this is occasionally due to the trabecular obstruction by the iris. The mode of insurgence of this type of glaucoma, unlike other forms, is very rapid and is therefore also known as acute glaucoma. In this condition one of the main risk factors is also associated to familial and/or ethnic factors. As a matter of fact, East asian populations, the Chinese one in particular, show a significant aptitude towards this pathology, other risk factors being the patient's age (above 50 years of age the incidence of the pathology increases) and hypermetropia. To date a decisive therapy for neither open nor closed angle glaucoma is available, however some treatments exist allowing the slowing, and in some cases the arrest, of the progression of the disease.

Secondary glaucoma may develop as a consequence of other pathologies such as inflammation, cataract, traumas, pigments released from the iris and, finally, tumors. In this situation the eye activates its defense producing the hyper-secretion of aqueous humor thus leading to ocular hypertension. One of the main characteristics of glaucoma is the increased excavation of the optic disk which extends towards its margins. Even though some studies support the idea that the pathology may start at retinal level, some indications exist that the early lesions occur at the level of the head of the optic nerve, in particular on the *lamina cribrosa*. Investigations demonstrate that the death of RGC occurs by apoptosis [1, 2]; the activation of this process, most likely, causes a reduction of the number of axons forming the optic nerve and this would evolve to the clinical signs consisting in the characteristic increase of papilla excavation which results in a reduction of the optic visual field. The ganglion retinal cells are the first target of the damage, mainly those found in the temporal region of the retina where the *lamina cribrosa* is thinner and thus gives an inefficient structural support to the RGC axons [3].

Hypotheses on the mechanisms of cell degeneration are diverse, the mechanical stress and the ischemic model being two of the most corroborated ones. The mechanical stress theory purports that the increase of the IOP within the anterior chamber causes a direct hyper-pressure at the retina-vitreous interface. This mechanical stress would directly trigger cell death by physical compression. According to this theory the mechanical insult causes modifications of the cell function: with respect to this, it has been reported that this type of insult may alter gene expression in organs such as the heart and the endothelial vessels. Furthermore, by the activation of transduction pathways, different functional responses are induced in retinal cells and astrocytes [4]. This IOP-induced mechanical stress could also inhibit the retrograde transport along the ganglion cell axons. Regarding this particular point, it has been observed a block in the axonal transport at the level of the *lamina cribrosa* followed by a drastic

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reduction of neurotrophins required for the survival of the RGC [5]. Furthermore, a reduction of the axon-plasma transport and the accumulation of toxic level of neurotransmitters have been observed; also, an increase of nitric oxide and endothelins as well as remodeling of the extra-cellular matrix has been monitored. Studies validate, on the other hand, the theory of the ischemic model, *i,e,* the vascular model of ischemia, as a main cause of the increased mechanical compression and subsequent oxidative stress at cell level.

The ischemic hypothesis postulates that the high intraocular pressure and the deformation of the *lamina cribrosa* may generate a compression of the blood vessels at retina and/or optic nerve level with a subsequent ischemic damage. In the pathological ischemic condition a temporary interruption of blood perfusion occurs and this determines a lack of oxygen, glucose and trophic substances in general. In patients with normal pressure and open angle glaucoma it was reported a decrease of the blood flow at the head of the optic nerve and an increase of hemagglutination. In addition, in this type of glaucoma an alteration of endothelin-mediated blood flow occurs. This protein is expressed in the endothelial cells and constricts blood vessels thus raising the blood pressure; its action is mainly exerted on the smooth muscles of the blood vessels [6]. The raise of the IOP plays a crucial role in the etiology of the disease, however the observation of glaucoma patients with normal pressure values suggests that diverse factors act synergistically to the insurgence of the pathology.

The glaucoma neuropathy may be also due to an insufficient vascular perfusion of the optic nerve which causes an ischemic damage to this organ. The ischemia thus generated, ends in an oxidative stress at RGC level and causes apoptotic death. This phenomenon happens because when re-perfusion initiates, the presence of oxygen in the tissue exposed to ischemia, induces the formation of radical oxygen species (ROS). When the concentration of ROS is too high, the anti-oxidant systems of the cell become unable to inactivate them, due to a deficient homeostasis, thus the free radicals are no longer neutralized and may cause cell death either via apoptosis or necrosis. In conclusion both types of stress, the mechanical and the ischemic one, can contribute to the establishment of the disease [2].

1.1. Cellular targets of the ocular hypertension

A complex interaction between neural and glial cells exists during the differentiation and the life of the nervous system. As a matter of fact, neuroglia cells maintain the normal functions of the nervous system since they control the extra cellular environment, block the toxic agents and supply the trophic resources and, last but not least, provide a structural support to the neurons. In glaucoma, astrocytes play a very important role as far as the re-modeling of the *lamina cribrosa* is concerning. Actually, they may also have a role also in the onset of the disease. Studies conducted on human glaucoma have, in fact, evidenced that the disorganization at astrocyte level in the anterior areas of the optic nerve, is associated to hypertrophy and over-expression of the glial fibrillary acidic protein (GFAP) which also occurs in astrocyte cultures subjected to high hydrostatic pressure. Following ischemic episodes, traumas or neuro-degenerative disorders, the phenotype of the astrocyte cells and microglia, activates the production of cytokines, ROS, nitric oxide and tumor necrosis factor α (TNF-α); all these molecules are mediators involved in the tissue damage [2]. In a similar way, glial cells located

in the retina and in the head of the optic nerve may carry out their normal physiological role as supporters of the cell bodies and their relative axons of the ganglion cells; on the contrary they may have a noxious role towards the same structures in pathological conditions.

1.2. Oxidative stress and retinal ganglion cell death in glaucoma

Oxidative stress is initiated by the imbalance between the production of ROS and their elimination by antioxidants. This phenomenon plays a key role in neuronal damage ending with neuron death which usually occurs by apoptosis. These reactive oxygen species are produced by mitochondria but can also derive from enzymatic degradation of neurotransmitters, neuroinflammatory mediators, and redox reactions [7]. Mitochondrial dysfunction can result in an increased level of ROS which is often found in neurodegenerative pathologies. Abnormal protein folding, defective ubiquitination and proteasome degradation systems may cause the production of ROS [8]. This promotes neuronal death *via* diverse molecular mechanisms including protein modification and DNA damage [9]. In any case, whether the oxidative stress triggers cell death is a component of a more complex neuro-degenerative process is yet to be elucidated [8]. Literature reports exist showing that neural damage occurs following oxidative stress in animal models of optic nerve injury and in human glaucoma. For example, DNA damage as well as protein and lipid peroxidation products, such as malonal-dihaldehyde accumulate in the trabecular meshwork and retina in animals with raised IOP [2, 10 - 16]. The high concentration of intra-cellular ROS has also been proposed as a crucial death signal after axonal injury, even though this may not directly cause a glaucoma, which would lead to RGC apoptosis [17 – 21]. Dysfunction of perfusion and reduced oxygen availability may play a role in the insurgence of an oxidative damage [22, 23]. The formation of ROS at mitochondrion level is required to activate a transcription factor known as hypoxia-inducible factor-1 alpha that induces the expression of several genes involved in the control of hypoxia, [24, 25]. Cells have a very effective protective antioxidant system including superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase [26]; if this systems partially or totally fail in neutralizing the ROS in the RGCs population, the progression of glaucoma could be triggered. Evidence exists supporting this idea; as a matter of fact SOD activity is lower than normal in the trabecular meshwork of glaucoma patients [27, 28] and in the retina as monitored in experimental ocular model of hypertension [19]. A recent study *in vivo* showed a dramatic increase in RGCs after optic nerve axotomy which preceded apoptosis [19]. Reactive oxygen species alter the redox equilibrium in the cell and this produces cysteine sulfhydryl oxidation. As a consequence oxidative cross-linking leads to the formation of new disulfide bonds that result in conformational changes of the proteins and activation of apoptotic signals [29, 30]. To date many studies have shed light on the molecular events causing the death of RGC. These evidences were gathered from investigations on animal models where acute or chronic optic nerve damage was generated and in experimentally induced glaucoma. A number of cellular phenomena are involved in the apoptotic death of RGCs; just to mention some: deprivation of neurotrophic factor, loss of synaptic connectivity, oxidative stress, axonal transport failure (for an exhaustive review on this topics see [31]).

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Apart from the elevated intraocular pressure, other risk factors such as genetic background, decreased corneal thickness, age and vascular dys-regulation may play an important role in the insurgence of glaucoma [32 - 39]. However, even if these factors may determine a risk to develop the disease, it remains difficult to establish a cause/effect relationship to develop this pathology: actually, one should consider that a high intraocular pressure is common among open-angle patients but many individuals showing this sign eventually will not develop glaucoma [40]. A further apparently paradoxical phenomenon is that a significant number of glaucoma patients progressively lose vision even though they react positively to drugs lowering the IOP [41 - 44]. In conclusion the cause of RGC in glaucoma still remains to be fully elucidated. Certainly the understanding of the apoptic death in RGC determined by the pathology is to be ascribed to the high complexity and the multifactorial character of the disease. The development of new neuroprotective therapies, even though will give a scant contribution to the elucidation of the molecular and cellular mechanisms underlying the disease, will certainly help to slow the development and progression of the pathology in glaucoma patients.

1.3. Mitochondrial malfunctions and ophthalmogical diseases

The association of ophthalmologic diseases to a mitochondrial etiology is assuming an increasingly interest: many authors consider, as a matter of fact, that the pathologies originate from impaired mitochondrial function, oxidative stress and enhanced apoptotic death. The mitochondrial role in the development of primary congenital glaucoma, characterized by trabecular dysgenesis, has been recently suggested. The formation of the trabecular meshwork during development is thought to have particular sensitivity to oxidative stress induced damage. Mitochondrial DNA (mtDNA) mutations, in particular, are emerging as causative agents of ophthalmologic disorders affecting mostly the optic nerve and the retina as well as the extra-ocular muscles. Also in these cases antioxidant therapy represents a good tool to treat these ophthalmologic conditions. Mitochondrial dysfunction is suggested, for example, to play an important role in age related macular degeneration, glaucoma and diabetes dependant retinopathy. Some biomarkers have been identified in the mitochondrial oxidative stress response: for instance, prohibitins also known as PHB may have diverse functions and are also involved in mitochondrial structure and functionality. These proteins present a ring-like structure with 16–20 alternating Phb1 and Phb2 subunits in the inner mitochondrial membrane [45]. The precise molecular function of the PHB molecular complex is not clear even though it has been hypothesized that they may have a role as chaperone for respiration chain proteins or as providers of a scaffold for the optimal mitochondrial morphology and function. Prohibitins have been demonstrated to stimulate cell proliferation both in plants and mammals such as rodents. As far as tissue re-modeling is concerned, the proteins of the matrix metalloproteinase (MMP) family could be a useful tool in gene therapy aimed at the protection/rescue of the RGCs. Therefore PHB and MMP could constitute an effective biomarker and/or a therapeutic target for ophthalmologic pathologies. (For a recent review see [46]).

2. A model of experimental glaucoma in rat

Several experimental animal models exist to investigate the ocular pathologies. In our laboratory we have developed a rat model of hypertension that mimics and reproduces the situation found in human glaucoma. This animal model will be briefly reviewed in the following sections [2].

2.1. Induction of the intra-ocular hypertension

To induce ocular hypertension *in vivo* [50] causing a condition of acute glaucoma in rat we injected in the anterior chamber of the right eye methylcellulose (MTC) suspended in physiological solution (the contra-lateral eye served as control). The IOP was monitored by tonometry. The hypertension induced by MTC was also performed in the presence of the antioxidant trolox [50]. The degree of animal sufferance was evaluated by the behavioral Irwin test and by the recovery of bodyweight. Ocular inflammation was assessed by the Drize test adapted to the rat, both approaches were described in detail in [49]. Intra-ocular pressure was monitored on 20 different animals that were finally sacrificed by hemorrhagic shock (decapitation). The eyes were removed and the cornea eliminated at limbus level; vitreous humor, and crystalline lens were discarded. The remaining samples of retina and optic nerve were fixed in paraformaldehyde, quickly washed in PBS finally included in freezing resin and cryostat-cut. Chromatin morphology and structure as well as DNA fragmentation was evidenced by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) reaction and validated by the formation of the apoptotic ladder after agarose gel electrophoresis. The apoptotic ladder is generated by nucleolytic inter-nucleosomal DNA cleavage since during the late stages of apoptosis the enzyme DNase I is activated. This causes the formation of multiple nucleosomal DNA fragments which can be easily visualized, by gel electrophoresis, by fluorescence after ethidium bromide staining.

2.2. Lipoperoxidative damage of the membrane and apoptosis after induction of cell stress

The data obtained in our laboratory support the idea that ocular hypertension causes apoptotic death of retinal ganglion cells and over-expression of molecular markers typical of oxidative cell stress response and apoptosis. Glial cells may have a neuroprotective role in a pathological situation; in any case they may contribute protection from neuron damage. In particular, during progression of glaucoma, astrocytes are involved in the re-modeling of the *lamina cribrosa* and they could act as initiators of the pathology. With respect to this see the role of PHB and MMP mentioned in preceding section. Studies on experimental models of ocular hypertension and human glaucoma evidenced an astrocyte hypertrophy and a loss of organization both at retina and optic nerve level. The up-regulation of the GFAP was also observed, as mentioned in a previous section of this work, in cultured astrocytes grown at high hydrostatic pressure. The GFAP is considered a very important stress marker in diverse retinal pathologies. Activation of the glial cells may also have noxious consequences on neurons, as they may cause mechanical damages and alterations of the micro-enviroment also, they may fail to provide the structural/nutritionl support to the neural cells. This could trigger the release

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and/or production of neurotoxic and proapoptotic compounds such as nitric oxide synthase (NOS). The nitric oxide thus produced is a reactive free radical present in cells as a response to increased intracellular concentrations of Ca^{2+}. It is known that NOS increases in cerebral ischemia and the over-expression of this enzyme causes relevant damage: the overall result is a detrimental action on the cell membrane. Recent studies demonstrated that an excess of NO is toxic and this compound increases as a consequence of ocular hypertension. In glaucoma, the involvement of inducible NOS (iNOS) has also been suggested. The oxidative stress and the increase of IOP also cause up-regulation of ubiquitin (Ub) and stimulation of the Ub-proteasome pathway: this possibly derives from the activation of the apoptotic program. In any case it should be pointed out that we also demonstrated that a well-known natural substance, carnitine, endowed of antioxidant properties and improvement of muscle performance, can ameliorate the glaucomatous pathology in the rat model system developed in our laboratory [2, 16].

3. Conclusions

In conclusion, literature data imply that the RGCs are one of the main targets of the oxidative stress in the neural tissue. As shown in our studies, the injection of methylcellulose into the anterior chamber of the eye activates diverse signals of stress at the level of RGCs. Mainly, the up-regulation of the GFAP and DNA damage become evident. Methylcellulose hinders the efflux of fluids from the canals of Schlemm thus increasing the IOP. The consequent oxidative stress is shown by the overexpression of iNOS, which is an enzyme primarily involved in the mitochondrial lipid peroxidation, with consequent damage of the cell membrane. This is validated by the accumulation of intracellular malonal-dihaldehyde: a hallmark of lipoperoxidation. The ubiquitin-mediated proteasome pathway is also activated and this is directly related to the execution of the apoptotic death. The antiapoptotic role of carnitine plays a key role in the stabilization and function of the cell membrane, the mitochondrial one in particular. The contemporary treatment with methylcellulose and carnitine reduces the level of typical markers of cell sufferance and apoptotis, this enhances the mitochondrial performance, improves the overall homeostatic response to the hypertensive insult, and limits the apoptotic phenomena.

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References

[1] Capaccioli, S, Nucci, C, Quattrone, A, & Carella, E. Apoptosi nel glaucoma. In: Apoptosi in oftalmologia, Ed I.N.C. (1998). , 44-57.

[2] Calandrella, N, Scarsella, G, Pescosolido, N, & Risuleo, G. Degenerative and apoptotic events at retinal and optic nerve level after experimental induction of ocular hypertension. Mol Cell Biochem, (2007). , 301, 155-163.

[3] Quigley, H. A, Dunkelberger, G. R, & Green, W. R. Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. Am. J. Ophthalmol (1989). , 107, 453-464.

[4] Wax, M. B, Tezel, G, Kobayashi, S, & Hernandez, M. R. Responses of different cells lines from ocular tissues to elevated hydrostatic pressure. Br. J. Ophthalmol (2000). , 84, 423-428.

[5] Quigley, H. A, Mckinnon, S. J, Zack, D. J, Pease, M. E, Kerrigan-baumrind, L. A, Kerrigan, F. D, & Mitchell, R. S. Retrograde axonal trasport of BDNF in retinal ganglion cell is blocked by acute IOP elevation in rat. Invest. Ophthalmol. Vis. Sci. (2000). , 41, 3460-3466.

[6] Morgan, J. E. Optic nerve head structure in glaucoma: astrocytes as mediators of axonal damage. Eye (2000). , 14, 437-444.

[7] Halliwell, B. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. (2006). , 97, 1634-1658.

[8] Andersen, J. K. Oxidative stress in neurodegeneration: cause or consequence? Nat. Med. (2004). , 5, 18-25.

[9] Cross, J. V, & Templeton, D. J. Thiol oxidation of cell signaling proteins: controlling an apoptotic equilibrium. J. Cell. Biochem. (2004). , 93, 104-111.

[10] Babizhayev, M. A, & Bunin, A. Lipid peroxidation in open-angle glaucoma. Acta Ophthalmol. (Copenh) (1989). , 67, 371-77.

[11] Izzotti, A, Saccà, S. C, Cartiglia, C, & De Flora, S. Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. Am. J. Med. (2003). , 114, 638-646.

[12] Ko, M. L, Peng, P. H, Ma, M. C, Ritch, R, & Chen, C. F. Dynamic changes in reactive oxygen species and antioxidant levels in retinas in experimental glaucoma. Free Radic. Biol. Med. (2005). , 39, 365-373.

[13] Moreno, M, Campanelli, J, Sande, P, Snez, D, & Keller-sarmiento, M. Rosenstein R Retinal oxidative stress induced by high intraocular pressure. Free Radic. Biol. Med. (2004). , 37, 803-812.

[14] Sacc, S, Pascott, A, Camicione, P, Capris, P, & Izzotti, A. Oxidative DNA damage in the human trabecular meshwork: clinical correlation in patients with primary open-angle glaucoma. Arch. Ophthalmol.(2005). , 123, 458-463.

[15] Tezel, G, Yang, X, & Cai, J. Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma. Invest. Ophthalmol. Vis. Sci. (2005). , 46, 3177-3187.

[16] Calandrella, N, De Seta, C, Scarsella, G, & Risuleo, G. Carnitine reduces the lipo-peroxidative damage of the membrane and apoptosis after induction of cell stress in experimental glaucoma. Cell Death Dis. (2010). e 62.

[17] Geiger, L. K, Kortuem, K. R, Alexejun, C, & Levin, L. A. Reduced redox state allows prolonged survival of axotomized neonatal retinal ganglion cells. Neuroscience (2002). , 109, 635-642.

[18] Kanamori, A, Catrinescu, M. M, Kanamori, N, Mears, K. A, Beaubien, R, & Levin, L. A. Superoxide is an associated signal for apoptosis in axonal injury. Brain (2010). , 133, 2612-2625.

[19] Lieven, C. J, Schlieve, C. R, Hoegger, M. J, & Levin, L. A. Retinal ganglion cell axotomy induces an increase in intracellular superoxide anion. Invest. Ophthalmol. Vis. Sci. (2006). , 47, 1477-1485.

[20] Nguyen, S. M, Alexejun, C. N, & Levin, L. A. Amplification of a reactive oxygen species signal in axotomized retinal ganglion cells. Antioxid. Redox Signal. (2003). , 5, 629-634.

[21] Swanson, K. I, Schlieve, C. R, Lieven, C. J, & Levin, L. A. Neuroprotective effect of sulfhydryl reduction in a rat optic nerve crush model. Invest. Ophthalmol. Vis. Sci. (2005). , 46, 3737-3741.

[22] Izzotti, A, Bagnis, A, & Sacc, S. The role of oxidative stress in glaucoma. Mutat. Res. (2006). , 612, 105-114.

[23] Tezel, G. Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. Prog. Retin. Eye Res. (2006). , 5, 490-513.

[24] Chandel, N. S, Mcclintock, D. S, Feliciano, C. E, Wood, T. M, Melendez, J. A, Rodriguez, A. M, & Schumacker, P. T. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. J. Biol. Chem. (2000). , 275, 25130-25138.

[25] Duranteau, J, Chandel, N. S, Kulisz, A, Shao, Z, & Schumacker, P. T. Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. J. Biol. Chem. (1998). , 273, 11619-11624.

[26] Vendemiale, G, Grattagliano, I, & Altomare, E. An update on the role of free radicals and antioxidant defense in human disease. Int. J. Clin. Lab. Res. (1999). , 29, 49-55.

[27] Behndig, A, Svensson, B, Marklund, S. L, & Karlsson, K. Superoxide dismutase isoenzymes in the human eye. Invest. Ophthalmol. Vis. Sci. (1998). , 39, 471-475.

[28] [28] De La Paz, M. A, & Epstein, D. L. Effect of age on superoxide dismutase activity of human trabecular meshwork. Invest. Ophthalmol. Vis. Sci. (1996). , 37, 1849-1853.

[29] Carugo, O, Cemazar, M, Zahariev, S, Hudaky, I, Gaspari, Z, Perczel, A, & Pongor, S. Vicinal disulfide turns. Protein Eng. (2003). , 16, 637-639.

[30] Park, C, & Raines, R. T. Adjacent cysteine residues as a redox switch. Protein Eng. (2001). , 14, 939-942.

[31] Almasieh, M, Wilson, A. M, & Morquette, B. Cueva Vargas J.L., Di Polo A. The molecular basis of retinal ganglion cell death in glaucoma. Prog Retin Eye Res (2012). , 31, 152-181.

[32] Agis, I. The advanced glaucoma intervention study (AGIS): 7. the relationship between control of intraocular pressure and visual field deterioration. Am. J. Ophthalmol. (2000). , 130, 429-440.

[33] [33] Gordon, M, Beiser, J, Brandt, J, Heuer, D, Higginbotham, E, Johnson, C, Keltner, J, Miller, J, Parrish, R. N, Wilson, M, & Kass, M. The Ocular hypertension treatment study: baseline factors that predict the onset of primary open-angle glaucoma. Arch. Ophthalmol. (2002). , 120, 714-720.

[34] Leske, M. C, Heijl, A, Hyman, L, Bengtsson, B, Dong, L, & Yang, Z. EMGT Group. Predictors of long-term progression in the early manifest glaucoma trial. Ophthalmology (2007). , 114, 1965-1972.

[35] Leske, M. C, Connell, A. M, Wu, S. Y, Nemesure, B, Li, X, Schachat, A, & Hennis, A. Incidence of open-angle glaucoma: the Barbados eye studies. The Barbados Eye Studies Group. Arch. Ophthalmol. (2001). , 119, 89-95.

[36] Mukesh, B. N, Mccarty, C. A, Rait, J. L, & Taylor, H. R. Five-year incidence of open-angle glaucoma: the visual impairment project. Ophthalmology (2002). , 109, 1047-1051.

[37] Wolfs, R. C. W, Klaver, C. C. W, Ramrattan, R. S, Van Duijn, C. M, Hofman, A, & De Jong, P. T. V. M. Genetic risk of primary open-angle glaucoma: populationbased Familial aggregation study. Arch. Ophthalmol.(1998). , 116, 1640-1645.

[38] Medeiros, F. A, Sample, P. A, Zangwill, L. M, Bowd, C, Aihara, M, & Weinreb, R. N. Corneal thickness as a risk factor for visual field loss in patients with preperimetric glaucomatous optic neuropathy. Am. J. Ophthalmol. (2003). , 136, 805-813.

[39] Leske, M. C. Ocular perfusion pressure and glaucoma: clinical trial and epidemiologic findings. Curr. Opin. Ophthalmol. (2009). , 20, 73-78.

[40] Friedman, D. S, Wilson, M. R, Liebmann, J. M, Fechtner, R. D, & Weinreb, R. N. An evidence-based assessment of risk factors for the progression of ocular hypertension and glaucoma. Am. J. Ophthalmol. (2004). , 138, 19-31.

[41] Caprioli, J. Neuroprotection of the optic nerve in glaucoma. Acta Ophthalmol. Scand. (1997). , 75, 364-367.

[42] Georgopoulos, G, Andreanos, D, Liokis, N, Papakonstantinou, D, Vergados, J, & Theodossiadis, G. Risk factors in ocular hypertension. Eur. J. Ophthalmol. (1997). , 7, 357-363.

[43] Harbin, T. S, Podos, S. M, Kolker, A. E, & Becker, B. Visual field progression in open-angle glaucoma patients presenting with monocular field loss. Trans. Sect. Ophthalmol. Am. Acad. Ophthalmol. Otolaryngol. (1976). , 8, 253-257.

[44] Leske, M. C, Heijl, A, Hussein, M, Bengtsson, B, Hyman, L, & Komaroff, E. For the Early Manifest Glaucoma Trial Group,. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. Arch. Ophthalmol. (2003). , 121, 48-56.

[45] Tatsuta, T, Model, K, & Langer, T. Formation of Membrane-bound Ring Complexes by Prohibitins in Mitochondria. Mol. Biol. Cell (2005). , 16, 248-259.

[46] Schrier, S. A, & Marni, J. F. Mitochondrial disorders and the eye. Current Opinion in Ophthalmology (2011). , 22, 325-331.

[47] Zhu, M. D, & Cai, F. Y. Development of experimental chronic intraocular hypertension in the rabbit. Austl Nw Z J Ophthalmol (1992). , 20, 225-234.

[48] Mcclain, D. E, Kalinich, J. F, & Ramakrishnan, N. Trolox inhibits apoptosis in irradiated MOLT-4 lymphocytes. FASEB J (1995). , 9, 1345-1354.

[49] Drize, J. H, Woodard, G, & Calvery, H. O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharm Exp Ther (1944). , 82, 377-390.

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