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Evidence-based pathophysiology of glaucoma

Cristina ALEXANDRESCU[1], Ana-Maria DASCALU[1], Costin MITULESCU[2], Aida PANCA[1], Ruxandra PASCU[1], Radu CIULUVICA[1], V. POTOP[1], Liliana Mary VOINEA[1]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]Clinic of Ophthalmology, Emergency University Hospital, Bucharest, Romania

Glaucoma, one of the major causes of blindness worldwide, is a chronic neurodegenerative disease of the optic nerve, which consists of progressive loss of the retinal ganglion cell fibers and visual field defects. High intraocular pressure has long been considered the most important risk factor for the onset and progression of glaucoma. Ever since, it was defined as a clinical entity (in the second half of the 19th century) and until a decade ago, treatment for glaucoma has focused on lowering intraocular pressure to stop progression. Yet, glaucomatous optic nerve damage progresses even when intraocular pressure is under control and, in normal tension glaucoma, optic disc changes and visual field defects appear while intraocular pressure is considered normal (1,2).

Progress made in the past few years in medical research has allowed a new approach to the pathophysiology of glaucoma, by studying the pathologic process on a tissue, cellular, molecular and genetic level.

Aim: to review the most relevant data regarding the pathophysiology of glaucoma published in the last decade. ❑

1. ROLE OF THE VASCULAR FACTOR

Some studies have shown a link between ocular perfusion pressure and glaucoma, as it is known that glaucoma can progress despite low intraocular pressure. *Costa and col* evidenced the importance of autoregulation to maintain the adequate perfusion of the optic nerve head, and suggest that ocular perfusion pressure and its fluctuation may be parameters that need to be measured in glaucoma patients. (3) Progression in case of normal tension glaucoma has been associated with deficiencies in the mechanism of regulation of ocular circulation. Interestingly, reduction of blood flow was also observed in the nail-fold capillaries of fingers in glaucoma patients suggesting that the reduction of blood flow is not due to increased IOP or an epiphenomenon of glaucoma, but a global vascular dysregulation is involved in POAG especially in NTG cases (4). Doppler im-

Address for correspondence:
Ana-Maria Dascalu, MD, "Carol Davila" University of Medicine and Pharmacy, 8 Eroilor Sanitari Blvd., Bucharest, Romania
email address: dr.anamaria.dascalu@gmail.com

aging of the ophthalmic artery has shown a different pattern in patients with low tension glaucoma, compared to normal individuals, with increased resistance to blood flow and systolic velocity increase. Researches have proved that the long term changes in retinal circulation can lead to glaucoma-like aspects of the optic disc, independent of intraocular pressure. Several abnormalities were found in the choroid, retinal and retrobulbar circulation of patients with chronic open angle glaucoma (5,6). With the development of Heidelberg Retina Flowmeter, it has become possible to obtain live images of the retinal circulation. *Logan and col*. demonstrate the relationship between glaucomatous damage and changes in the pattern of retinal circulation and suggest that the latter could be an early marker for the progress of the disease. (7). This study supports the idea that endothelial lesions and atherosclerosis (due to age, inflammation, reduced blood flow) can lead to damage of the retinal nerve fiber layer and the underlying conjunctive tissue. This regulation of the blood flow differs in different ocular tissues. The retinal vascular regulation is similar to that of the brain except the fact that it has no autonomic innervations.

Among other vascular risk factors, there are some theories which support the pathogenic role of endothelial dysfunction. Primary endothelial dysfunction can affect the diameter of blood vessels and can lead to increased flow resistance. Endothelial cells produce two types of molecules: endothelin 1, a vasoconstrictor, and nitric oxide (NO), a vasodilator.

Blood flow of the retina is largely regulated by the endothelial cell derived substances that are collectively known as endothelium derived vasoactive compounds (EDVCs), acting both abluminally and intraluminally. Patients with primary vascular dysregulation often have high level of endothelin-1 (ET-1) (8, 9). ET-1 has the capability of vasoconstriction and also interferes with vascular permeability. By increasing the vascular permeability it can result in retinal hemorrhage that has also been observed in glaucoma patients, especially in NTG cases (10). The vascular leakage can facilitate the diffusion of harmful materials to cross the incomplete blood brain barrier in the retina. Apart from vasoconstriction, ET-1 also regulates the blood brain barrier, by upregulating the prostaglandin E2 which in turn reduces the endothelial tight junction complex (Grieshaber and Flammer 2007). ET-1 cause ischemic stress not only by inducing vasoconstriction but also by altering the activity of ATP dependent Na+/K+ pump (11).

Nitric oxide (NO) is an important messenger intra and extra molecular implicated in vasodilatation, contractility, neurotransmission, neurotoxicity and inflammation. NO is formed from L-arginine by nitric oxide synthase (NOS). Nitric oxide synthase has three isoforms: NOS-1 neuronal, NOS-2 inducible and NOS-3 endothelial (role in vasodilatation). Nitric oxide has a demonstrate role in many neurodegenerative diseases like: glaucoma, Alzheimer disease, multiple sclerosis and cerebral-cardiovascular diseases. Nitric oxide (NO) has more than one role in inducing glaucoma. It is tissue soluble and diffuses along membranes due its small size. NO is unstable and has a short life span. Its biological markers are nitrites and cyclic guanosin monophosphate (cGMP).

NO, as suggested by the vascular pathogeneses theory of glaucoma, is responsible for counter-balancing the vessel-tone increase (8, 10). But it also plays an important role in neuronal physiology by acting as a second messenger and by modulating the cellular sodium pump. Through these mechanisms, NO increases the production of glutamate and other intercellular messengers, which in turn cause a marked and prolonged alteration in activity of the ATP dependent, Na+/K+ pump, a mechanism implicated in various degenerative diseases (9), including glaucoma (12,13).

Different isoforms of NO-Synthetase explain the multiple roles of NO in inducing glaucoma. NO produced by the eNOS (endothelial nitric oxide synthase) has different metabolic roles from the NO produced by NOS (nitric oxide synthase) located in the trabeculum, the latter not being responsible for the production of free radicals, such as peroxinitrites, potentially toxic for ocular structures. The trabecular distribution of NOS suggests an important role of nitric oxide in the future therapies for the glaucoma. The increase of nitric oxide makes vasodilatation and improves contractility in the trabecular meshwork; the final effect being the decrease of intraocular pressure and on the

other hand the contra-apoptotic effect giving neuroprotection. (14). More than that, recent studies proved that a topical nitric oxide-releasing dexamethasone (NCX1021) may avoid the negative effects of dexamethasone phosphate, such as the IOP increase, impairment of ocular blood flow and the morphological changes in the ciliary bodies possibly induced by corticosteroid treatment. This fact could represent a therapeutic solution for glaucomatous patients who need topic corticosteroid theraphy (15). ❑

2. GLAUCOMA AND NEURODEGENERATIVE DISORDERS (PARKINSON'S AND ALZHEIMER'S DISEASES)

Recent findings, no longer consider glaucoma as an autonomous dysfunction, affecting a single population of cells— the retinal ganglion cell fibers. More and more data suggest that glaucoma should be integrated in the category of neurodenerative diseases, the mechanisms involved in cell degeneration and neuron death are very similar to those in Parkinson's and Alzheimer's diseases. There are also clinical and pathological data implying that glaucoma patients also have cerebral degenerative lesions of the optic nerves, lateral geniculate bodies and visual cortex.

Excitotoxicity is the process of neuronal damage due to excessive stimulation of the aminoacid-receptors. This process was at first discovered in the retina and has since been noticed in other ischemic or traumatic conditions of the central nervous system. Experimental studies search the role of memantine in treating glaucoma, an anti-excitotoxic drug used in Alzheimer's and the dementia associated with Parkinson's disease (1,2,16). ❑

3. OXIDATIVE STRESS OF THE RETINAL GANGLION CELL LAYER

Present researches aim to understand the mechanisms of survival, adaptation and death of retinal ganglion cells in order to discover the factors leading to lesions as well as the factors which protect these cells.

It has been proved that the death of retinal ganglion cells in glaucoma occurs through apoptosis. It is thought that increased oxidative stress, due to high levels of free radicals, can induce apoptosis of retinal ganglion cells and is thus involved in the pathogenesis of glaucomatous optic neuropathy (17,18).

Oxidative stress and antioxidative status of the ocular tissues have been assessed in a study conducted by Ferrerira and co. Total reactive antioxidant potential (TRAP) and the activity of antioxidizing enzymes: superoxide bismutasis, catalase and glutation peroxidase were measured. Results showed that TRAP was significantly lower in glaucoma subjects compared to the control group and that bismutasis and glutation peroxidase activity were higher, while catalase levels were the same. Therefore, oxidative stress can induce antioxidizing enzymes and can contribute to the lowering of TRAP. Superoxide bismutasis, glutation peroxidase and TRAP can be used as markers for oxidizing stress in patients with glaucoma (19,20). ❑

4. ROLE OF SEROTONIN

Serotonin is a neurotransmitter synthesized in neurons and deposited in intracellular vesicles. Serotonin is present in high amounts in the iris-cilliary body complex and seems to play a part in regulating the flow of the aqueous humor. Seven types of serotoninergic receptors have been identified (from 5-HT1 to 5-HT7). Stimulation of 5-HT7 leads to an increase of intraocular pressure, while stimulation of 5-HT1 leads to decrease in intraocular pressure (21-23).

Serotonin is also a precursor for melatonin, which plays an antixodizing role, can decrease IOP and lowers the level of NO. A study coordinated by Zanon-Moreno and co proved that chronic open angle glaucoma patients had low levels of serotonin and melatonin and high levels of 5-HIAA (5-hydroxyindoacetic acid, a product resulting from the degradation of serotonin) in the aqueous humor (24,25). ❑

5. PHYSIOPATHOLOGICAL ALTERATIONS OF THE AQUEOUS HUMOR AND THE TRABECULAR MESHWORK

a) transmembrane glycoprotein CD44 and hyaluronic acid

The aqueous humor contains proteins secreted by the anterior segment tissues and these proteins could play a significant role in inducing glaucoma.

Transmembrane glycoprotein CD44 is a surface cellular receptor for hyaluronic acid (HA), very frequent in ocular tissues and fluids. CD44 connects with growth factors and metaloproteases, thus playing a part in cellular growth processes and presenting enzymes to their substrates. CD44 is also necessary in activating receptors with high affinity (implicated in erbB2 phosphorylation and erbB2-erbB3 heterodimerization) vital to cell survival. Proteolitical cleavage of the extracellular domain of CD44 by matrix associated metaloproteases liberates sCD44 which plays different biological functions to intact CD44. sCD44 bioavailability depends on connecting to hylauronic acid, which is influenced by pressure. In the normal eye, HA connects to and inactivates sCD44. In COAG, the concentration of HA decreases in the aqueous humor and in the trabecular meshwork, and the level of sCD44 increases to double the normal value (26,27). Budak coordinated a study which showed that sCD44 levels were significantly higher in the aqueous humor of patients with COAG compared to normal or degenerative myopic individuals without glaucoma (28). Once the concentration of sCD44 reaches a threshold, the molecules become cytotoxic for some cells (trabecular meshwork cells, retinal ganglion cells, support cells in the initial segment of the optic nerve).

Knepper and col. suggested the hypothesis that changes in the levels of HA due to a rise in pressure and a decrease in the bonding of sCD44 with HA, can explain in part why high intraocular pressure is a risk factor for COAG. There are studies which show that exogenous sCD44 affects the survival of retinal ganglion cells and of trabecular meshwork cells in vitro by activating proapoptotic processes. Knepper has also showed in another study that, in the aqueous humor of COAG patients, the sCD44 molecule is hypophosphorylated compared to the aqueous humor of normal subjects, which induces a greater toxicity and a lower affinity towards hyaluronic acid, thus hypophosporylation of the sCD44 molecule could be a physiopahtological finding specific to COAG. Therefore sCD44 could be used as a marker protein in COAG (29,30).

b) TGF-beta2

Other molecules present in the aqueous humor are the transformation and growth factor beta-2 (TGF-beta2) and transthyretin. *Ozcan and col* did a study on a small number of subjects, and measured the level of TGF-beta2 in the aqueous humor in their study, *Grus and col.* measured the level of transthyretin in the aqueous humor, and results showed an increase in the levels of TGD-beta2 and thransthyretin in the aqueous humor of COAG patients, compared to the control group, which suggests that TGF-beta2 and transthyretin could play a part in the pathogenesis of glaucoma. A possible mechanism for transthyretin to induce glaucoma could be explained by the fact that the latter can form amyloid deposits which can obstruct the drainage of aqueous humor and lead to increased intraocular pressure (31, 32).

c) TNF alfa

Recent studies have suggested that TNF-α could play a role in the pathogenesis of glaucoma. *Hideko and col.* (33) measured the levels of TNF-α of patients with different types of glaucoma and compared them to a control group in their study. TNF-α was significantly higher in patients with glaucoma, a bigger difference was observed in patients with exfoliative glaucoma (29.6%), compared to COAG (13.7%) and low tension glaucoma (10.7%). Results suggest that TNF-α plays a key role in the progression of glaucoma.

d) Matrix metalloproteinases and integrins

In a different study conducted by Maatta, extracellular matrix metabolism through matrix metalloproteinases and their tissue inhibitors TIMP, was observed. The levels of MMP-2 and TIMP-2 were measured through ELISA. MMP had a low collagenolytic activity in both the glaucoma group and the control group, while the TIMP levels were significantly higher in glaucoma subjects. In conclusion, it is the accumulation of extracellular matrix rather than its degradation that prevails in COAG (34).

In the study conducted by Alvarado, endothelial cells of the Schlemm canal (SCEs) were brought into contact with endothelial cells of the trabecular meshwork (TMEs) which had previously been activated by laser light and the result was that the former exhibited 1120 genes for control (35).

Endothelial cells of the Schlemm canal exposed to laser exhibit only 12 genes. There are

two processes which suggest that cytokines interfere with these responses: specific cytokines produced by TMEs exposed to laser bonded with the SCEs and increased their permeability, and inactivation of TMEs by boiling or dilution eliminated this effect. This new mechanism, controlled by TMEs, is important for the pathogenesis of glaucoma and the mechanism through which laser trabeculoplasty works. Ligands identified to regulate permeability of the SCE can be used to treat glaucoma.

e) Modulation of regulation processes at the level of actin filaments of the cytoskeleton of trabecular meshwork cells

Experimental studies have proved that numerous molecules can interfere with the regulation process of the trabecular meshwork cells' cytoskeleton and the expression of adherens junctions between cells and with the extracellular matrix. An increase in actin branching in the cytoskeleton leads to increased resistance to flow, interfering with drainage of the aqueous humor through the trabecular meshwork.

Matrix proteases and integrins are important in the regulating pathways of the trabecular meshwork cellular cytoskeleton. In "in vitro" studies TGF beta induced stress on the actin filaments, making them more contractile. Myocilin and fibronectin also affect the trabecular cells. Mutant myocilin seems to lead to a decreased life span of the trabecular cells, leading to senescence, accumulation of the effects of oxidizing stress and possibly chronic open angle glaucoma (36).

f) atrial natriuretic peptide

Neuroendocrine regulation of the homeostasis of liquids in the body depends in part on the activation of the natriuretic system. It has been suggested that ocular natriuretic peptides contribute to the regulation of the dynamics of the aqueous humor. There are three types of natriuretic peptides: ANP-atrial NP, BNP-brain NP, CNP-C-type NP.

Brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) are cyclic endopeptides which mainly cause natriuresis and vasodilation. Administering ANP intravenously in animals and humans led to a decrease in IOP. J. Salzmann, in his case-control study, observed through radioimmunoassay the peptides in the aqueous humor of a group of patients with glaucoma and one without glaucoma, and the results confirmed the presence of BNP and ANP in the human aqueous humor, BNP was higher in the control group, while ANP was higher in glaucoma patients (37).

Some studies have shown that ocular hypotensive medication such as opioid K receptor agonists bremazocine and imidazoline-1/α2 agonist increase ANP in the AH in rabbits. It was concluded that natriuretic peptides released by medication from the cilliary epithelium cells can act as an autocrine or paracrine factor to modify intraocular pressure.

A study done by *Potter and col.* showed that bremazocine (BRE), a selective agonist of the K opioid receptor (KOR) reduces IOP in rabbits, especially by increasing the level of natriuretic peptide in the aqueous humor and by increasing the total elimination facility (TOF). The natriuretic peptide was inhibited by prior administration of norbinaltorphimine (KOR antagonist) or chelerythrine (protein kinase C inhibitor), and TOF was inhibited by administering norbinaltorphimine or isatin (R antagonist of the natriuretic peptide). These results showed that total elimination facility was increased by administering BRE, due to the paracrine effect of NP on tissue KOR along the pathway of elimination of the aqueous humor (38).

Takashima and col. demonstrated in a study that administering natriuretic peptides intra vitreally led to an increase in the ease of drainage of aqueous humor. This and *Fernandez-Durangosi and col.* showed that intravitreal injection of CNP was more efficient in lowering IOP than ANP and BNP (39, 40).

Further studies suggest that CNP has also a neuroprotective effect on rat retinal ganglionar cells. Toxic effect related to intravitreal injection of NMDA (20 nanomolecules) were significantly alleviated ($p < 0.05$) by concomitent injection of CNP (4.5 nmol, 10 μg). The neuroprotective effects of CNP were maintained up to 14 days after CNP injection (41). ❑

CONCLUSION

Glaucoma is a disease with complex physiopathogenic mechanisms, not entirely known. Treatment is based mainly on the reduction of intraocular pressure, which is considered the

main risk factor. Actual researches aim to elucidate the mechanisms involved in the survival, adaptation and death of retinal ganglion cells in hopes of uncovering factors which cause damage to and factors which protect these cells. New discoveries might contribute to the development of effective therapeutic means for protecting retinal nerve cells and counteracting the physiopathologic processes involved in glaucoma. ❑

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