Important Problems in Glaucoma and New Strategies in Therapy: Neuroprotection and Ocular Surface

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ABSTRACT

Glaucoma is a multifactorial, neurodegenerative disease which results in loss of vision if left untreated. Although elevated intraocular pressure (IOP) is the major, modifiable risk factor, there are some cases progressing despite effective IOP control. Again, there are also cases with marked optic nerve damage despite IOP considered to be within normal limits. This indicates that there are additional mechanisms of damage independent from elevated IOP in glaucoma. It will be possible to develop novel therapeutic strategies by better understanding of cellular problems and mechanisms of damage in glaucoma.

In the glaucoma, ocular surface disease is another problem with marked influence on treatment success. This may be due to both dry eye disease and antiglaucomatous molecules used in the treatment and preservative agents in the drug. This should be taken into account when planning medical treatment.

The current treatment in the glaucoma can be built as a triple strategy: IOP control, neuroprotection and solution of ocular surface problems. Here, we will address mechanisms of damage and related problems in the light of current knowledge and attempt to highlight remarkable molecules that are supportive in neuroprotection and ocular surface disease.

Keywords: Glaucoma, Ocular surface disease, Intraocular pressure, Apoptosis, Oxidative stress, Neuroinflammation, Neuroprotection, Brimonidine, ROCK inhibitors, Antioxidants, Coenzyme Q, Nicotinamide, Citicoline, Omega-3, Resveratrol, Curcumin, Magnesium.

Abbreviations: POAG: primary open-angle glaucoma, NTG: normal tension glaucoma, OHT: ocular hypertension, ALS: amyotrophic lateral sclerosis, OSD: ocular surface disease, RGC: retinal ganglion cell, NMDA: N-methyl-D-aspartate, ROS: reactive oxygen species, NO: nitric oxide, NOS: nitric oxide synthase, TNF: tumor necrosis factor, FasL: Fas-ligands, ET1: endothelin 1, TRX: thioredoxin, PGC-1α: peroksisome proliferator-activated receptor-γ co-activator 1α, NF-KB: nuclear factor-kappa B, IL: interleukin, JAK/STAT: janus kinase/signal transducer and activator of transcription, HSP: heat shock protein, NAM: nicotinamide, NAD: nicotinamide adenine dinucleotide, NR: nicotinamide riboside, Nmnat: nicotinamide mononucleotide adenylyl transferase, NGF: neuronal growth factor, BDGF: brain-derived growth factor, CNTF: ciliary neurotrophic factor, GDNF: glial cell neurotrophic factor, Trk: tropomyosin-kinase, ROCK: Rho-associated kinases, PARP: Poly (ADP-ribose) polymerase, SIRT: sirtuin, BAC: benzalchonium chloride, FGF: fibroblast growth factor, VEGF: vascular endothelial growth factor, SLN: solid lipid nanoparticle, Mg: magnesium.

INTRODUCTION

Glaucoma is a neurodegenerative disorder which progresses with RGC damage and axonal degeneration. Retina and optic nerve are parts of central nervous system. Degeneration and atrophic areas have been detected at lateral geniculate body on MR images from glaucoma cases.[1] It is thought that the glaucoma progresses with a pathogenesis similar to those in other degenerative diseases (e.g. Alzheimer's disease, Parkinson's disease, ALS). The neurodegeneration threatens visual system.[2, 5]

Many studies have been conducted in different models of glaucoma in order to elucidate pathophysiology in glaucoma. Based on current data, the mechanisms implied in RGC damage include: High IOP, excitotoxicity,

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Received: 26.10.2022
Accepted: 15.11.2022
J Glau-Cat 2022; 17: 155-165
DOİ: 10.37844/glau.cat.2022.17.26

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oxidative stress, reactive oxygen species and mitochondrial dysfunction, neurotrophin deficiency, immunomodulation disorder, neuroinflammation, vascular insufficiency and ischemia.[2-5] Genetic factors should also kept in mind.

The primary goal is to control IOP in glaucoma therapy. However, molecules which may diminish RGC damage and support neuroprotection should also be considered. In addition, ocular surface problems are another factor which may influence on patients compliance and treatment success.[6, 7] It is of important to solve ocular surface-related problems in patients with glaucoma.

Mechanisms of Retinal Ganglion Cell Damage in Glaucoma

Intrinsic (Excitotoxicity)-Extrinsic Apoptotic Pathways and Oxidative Stress

Apoptosis is programmed cell death. It advances through intrinsic and extrinsic pathways. In the process of neuronal cell degeneration, these pathways interfere with each other and convene at caspase-3 activation step. The increased glutamate is the major molecule which triggers intrinsic pathway. The glutamate released to inter-neuronal space is normally taken by Muller cells and converted to glutamine by glutamine synthase. However, glutamine cannot be removed from media when it is substantially released in stressed cells disrupted by various stimuli (elevated IOP, ischemia etc.) and in cells under stress, triggering excitotoxicity. It was found that intercellular glutamine concentration is increased in the glaucoma. This stimulates NMDA receptors on cell membrane, leading excessive Ca^{+2} influx into cell. It induces cytochrome C release from mitochondria and activation of caspases (caspase 7, 8, 9 and 3) while it leads substantial amounts of NO synthesis from arginine by stimulation of NOS pathway. The ROS (O_2^-, H_2O_2, OH) resulting from oxidative stress and excessive NO synthesis through NOS pathway leads production and accumulation of molecules, namely peroxynitrite and nitrotyrosine, highly toxic for neuronal cells. As a result, cell death occurs via chromatin condensation and DNA fragmentation.[2-5] FasL and TNF-α play role in the extrinsic pathway. They activate caspases by binding to death receptors (Fas, TNFR). Initially, caspase 8 is activated; followed by caspase 3 and 7 activation. The increased Ca^{+2} influx by excitotoxicity induces calcium-dependent catalytic enzymes, calcineurin and calpain. In this setting, cyclin-dependent kinase 5, stress-activated protein kinase and glycogen synthase kinase 3 are activated in the process where cytoskeleton is degraded while Bad gene, a mitochondrial membrane-bound proapoptotic gene, is dephosphorylated. Then, protein complexes including Bcl-2 and Bcl-X_L are formed; the permeability of mitochondrial membrane is increased and cytochrome C is released. As a result, apoptosis occurs through caspase activation (caspase 8, 9 and 3) and degradation of nuclear content by apoptosome formation.[2, 5]

Apoptotic/Anti-Apoptotic Genes and Mitochondrial Dysfunction

The effects of impaired balance between apoptotic (Bac, Bad, Bid) and anti-apoptotic genes (Bcl-2, Bcl-X_L) on mitochondria play an important role in the apoptosis. In such interaction, apoptotic gene activation triggered by several factors leads increased mitochondrial permeability, cytochrome C release from mitochondria and caspase activation.[2-5] RGCs are highly metabolic cells and require sufficient amounts of ATP which depends on proper functioning of mitochondria.[8] However, mitochondrial dysfunction develops due to above-mentioned mechanism and ROS effect as well. ATP production is interrupted. PGC-1α is one of the key molecules for mitochondrial bioenergetics. Its importance has been emphasized in many mitochondrial dysfunction related disorders (neurodegenerative diseases, cardiovascular diseases and cancer). It is thought that PGC-1α plays an important role in cellular energy generation in the glaucoma.

Vascular Dysregulation-Ischemia

In the glaucoma, vascular dysregulation and ischemia are other mechanisms underlying RGC damage. In case of prolonged hypoxia, cellular regulation mechanisms are completely disturbed. The redox system is collapsed, resulting increased ROS and free radicals.[11] The antioxidant molecules (glutathione, ascorbic acid) and antioxidant enzyme systems (superoxide dismutase, catalase, glutathione peroxidase) become deficient in the process. The protein, lipid and nucleic acid content of the cell is disrupted. At a point where process becomes irreversible, apoptosis occurs through p53 gene activation. NO-prostacyclin(vasodilatation)/endothelin (vasoconstriction) homeostasis is important in maintaining vascular regulation; the impaired homeostasis may predispose several disorders.

ET1 is a potent vasoconstrictor peptide. It is synthesized at vascular endothelial cells. IT induces vasoconstriction by increase in Ca^{+2} release from endoplasmic reticulum; this is mediated by inositol triphosphate (IP3) and diacylglycerol through activation of specific ET1 receptors. The ET1 and its receptors are extensively expressed in ocular tissues (iris, ciliary body, trabecular network). The ET1 leads IOP elevation by reducing trabecular flow; in addition, it plays role in RGC loss and development of glaucomatous damage by influencing on ocular perfusion. Increased ET1 levels in humor aqueous have been reported in various types of glaucoma.[9, 10] TNF-α enhances ET1 release, exerting some

deleterious effects through ET1. Figure 1 show a scheme for mechanisms of RCG damage.

Neuroinflammation

Neuroinflammation can be defined as any response against immunity caused by different neuronal cells, e.g. microglia, astrocyte and peripheral neuronal cells. Microglias are the immune cells of the central nervous system which initially respond to injury. They are involved in maintenance, protection and restoration of homeostasis in central nervous system. Thus, they are highly vital for RGCs. They undergo morphological conversion from ramified from to ameboid-phagocytic form when activated. They play important roles including release of neurotrophic factors and removal of cellular debris. However, in case of chronic activation (e.g. elevated IOP in glaucoma) which leads stress in neuronal cells and abnormal stimuli, they trigger inflammatory pathways together with oxidative stress. Astrocytes and Muller cells are also stimulated. The neuroinflammatory state develops through extensive cytokine release (IL1, IL6,TNFα) and NF-KB which is the major transcription factor in apoptosis.[11, 12] This neuroinflammatory state affects RGCs via JAK/STAT receptors and threatens RCCG viability. If neuroinflammatory state persists, RGC death is inevitable. Figure 2 depicts neuroinflammatory process.

Immune Modulation Disorder

In glaucoma, it is thought that immune modulation disorder developed due to T cell and complement activation as well as auto-antibodies is another pathway involved in RGC damage. Uncontrolled activation glial cells and cytokine

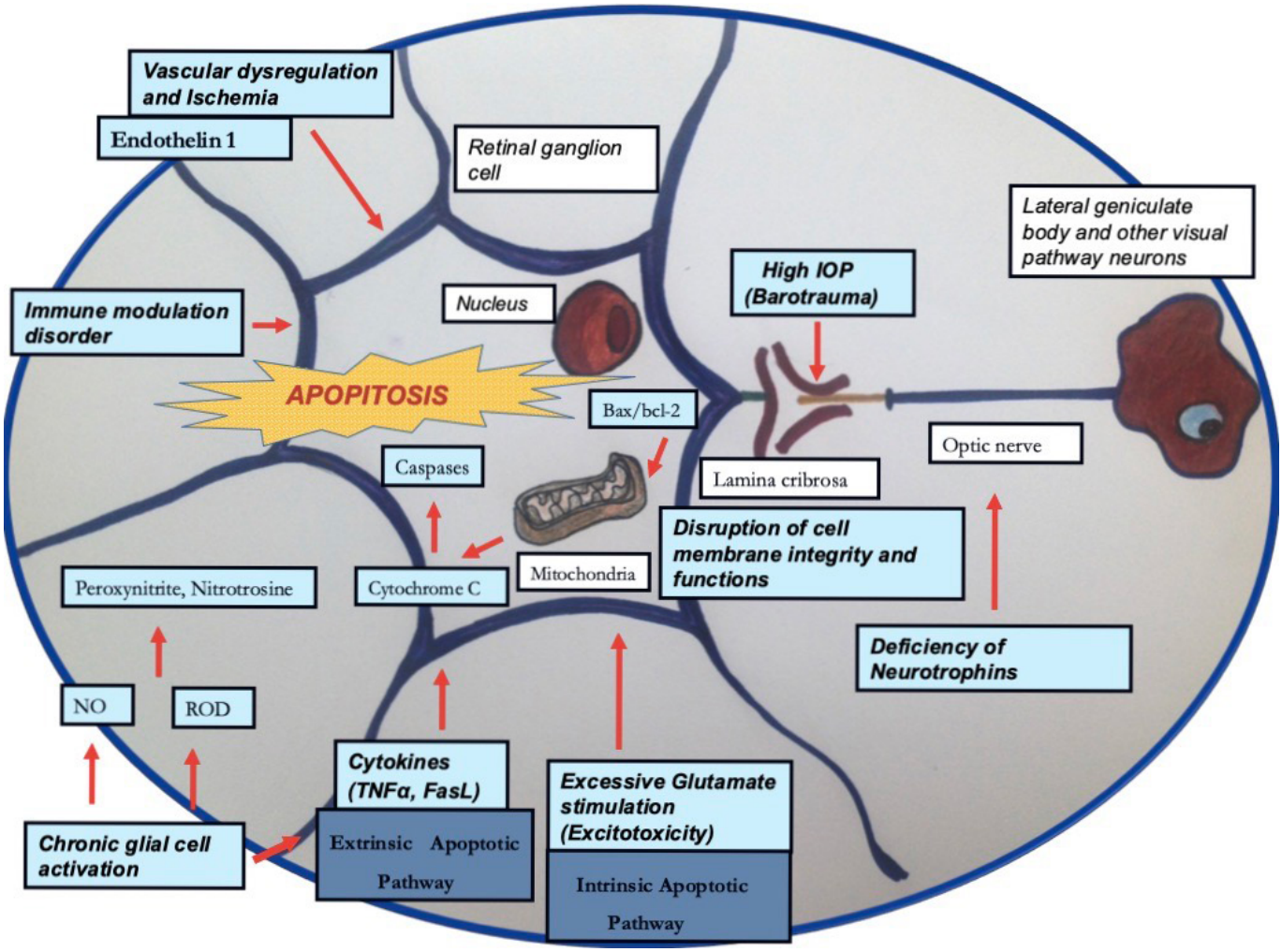

Figure 1: *Intra- and extra-RGC events and progression mechanism of damage are summarized schematically in glaucoma. The barotrauma due to elevated IOP, vascular dysregulation-ischemia and increased ET1 level, oxidative stress and mitochondrial dysfunction, impaired immune modulation, excessive glutamate stimuli (excitotoxicity), glial activation-cytokine release (TNFα, FasL), neuroinflammation, and neurotrophin deficiency play role in RGC damage. Cytokines are responsible in extrinsic apoptotic pathway while NDMA receptor-mediated excessive glutamate in intrinsic apoptotic pathway. In cell degradation process, caspase system is activated while mitochondrial activation by apoptotic genes and ROS effects increase membrane permeability, resulting in cytochrome C release. Thus, apoptosis becomes inevitable for RGC.*[4]

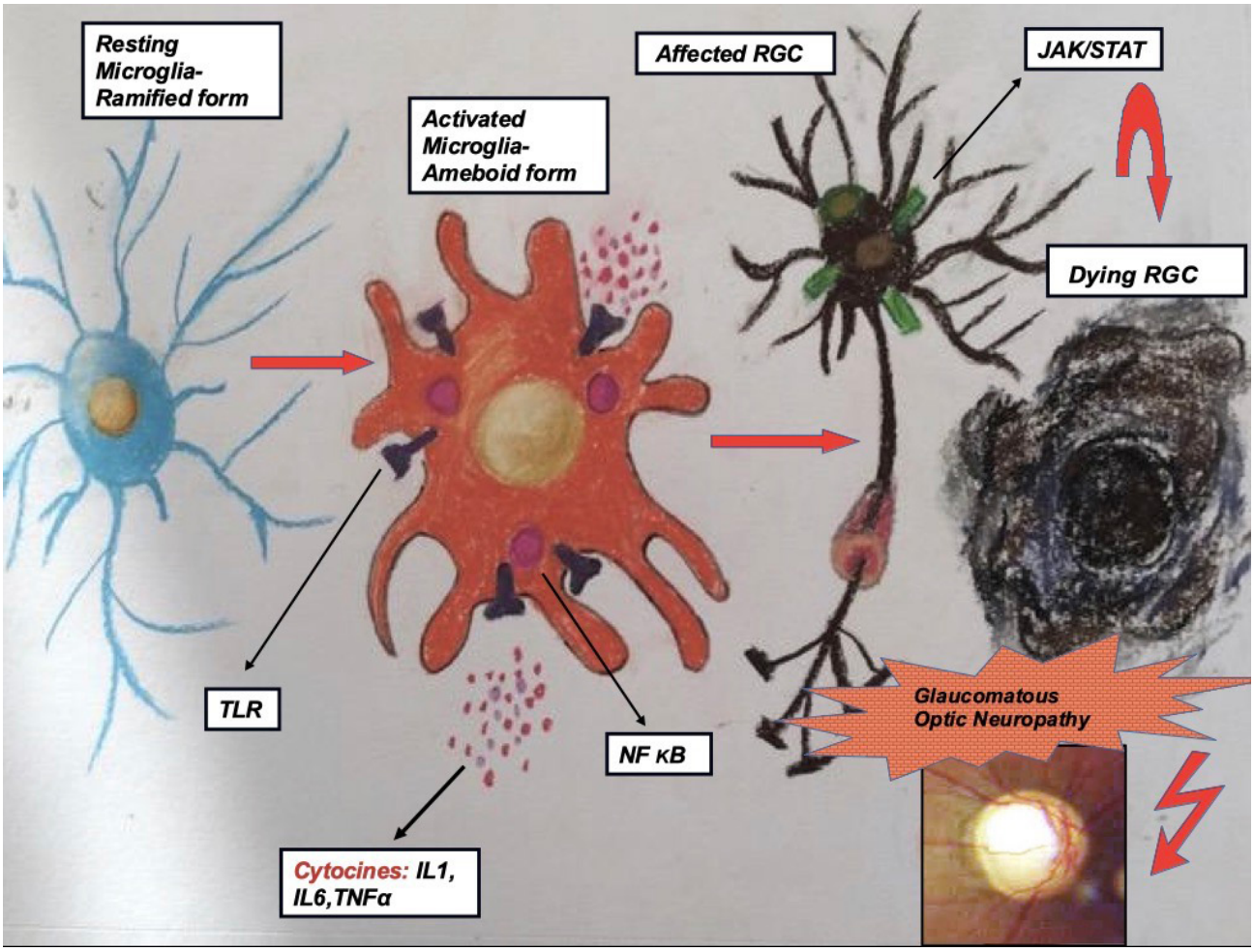

Figure 2: *Glial cell stimulation and neuroinflammation development are summarized schematically. Elevated IOP leads chronic glial activation by abnormal stimulation of neuronal cells, resulting in neuronal cell stress. By oxidative stress, inflammatory pathways are triggered and astrocytes and Muller cells are too stimulated in this process. The neuroinflammatory state develops through extensive cytokine release (IL1, IL6,TNFα) and NF-KB which is the major transcription factor in apoptosis. Presumably this neuroinflammatory state affects RGC via JAK/STAT receptors and threatens RCC viability. If this state persists, RGC death is in evitable.*[11]

release also play an important role in this pathway. It is though that HSP and myelination-related proteins activate immune system due to their antigenic nature. HSPs are released in stress conditions (hypoxia, temperature changes, nutritional problems). The increased HSP 60 and HSP 27 were shown in eyes with glaucoma. However, it hasn't been fully elucidated how these proteins function in glaucoma.[4, 13]

Deficiency of Neurotrophins

The neurotrophins (NGF, BDGF, CNTF, GDNF, NT-3,4,5) have important roles in development, differentiation and survival of RGCs. The biological effects of neurotrophins are mediated by 2 receptors: Trk A, B, C (tropomyosin-kinase) and p75 (p75^{NTR}). Trk receptor activation is associated to cell survival. It is thought that the neurotrophin deficiency is one of the mechanisms underlying neuronal damage in glaucoma.[4, 5]

Currently Remarkable Molecules on the Neuroprotection

In glaucoma management, there are cases who achieve effective IOP control by medication, laser therapy or surgery but show progression. In addition, in cases with NTG, disease progression may continue despite sufficient IOP reduction with therapy. The consistent questions from glaucoma patients regarding supportive therapy and commitment to reach accurate and potentially effective molecule have motivated us to make efforts to develop novel treatment strategies in glaucoma. Definitely, novel neuroprotective agents will be added to current molecules by growing interest on neuroprotection and exponential increase in the number of studies in this field.

Given the characteristics of ideal neuroprotective molecule in glaucoma as defined by Weinreb, the molecule should have specific receptors in retina and optic nerve; it should

be able to reach pharmacologically effective concentration in tissues; its the neuroprotective effect should have to be shown in experimental models; and finally, neuroprotective effect can be confirmed in randomized controlled clinical trials.[4] Given that the molecules shown to have neuroprotective effect in in vivo and in vitro studies may fail in phase trials (e.g. memantine), it is apparent that the process to develop novel agents is challenging. However, there are molecules with ongoing phase studies today.

In this section, we will review some remarkable molecules in neuroprotection field in the light of above-mentioned mechanisms, attempting to determine a road map.

It is though that **Brimonidine** exerts neuroprotective effect through multiple mechanism including anti-apoptotic gene activation (Bcl-2), controlling excitotoxicity due to glutamate, modulation of NMDA receptor functions and enhancing neurotrophin release.[4] Its neuroprotective effect has been confirmed in experimental studies using glaucoma models.[14, 15] In addition, in a multicenter trial comparing brimonidine and timolol in NTG cases, no significant difference was detected in IOP reduction but a significant difference was observed in the effect on visual field between two molecules, with a lower progression rate in visual field in cases treated with brimonidine (9.1% vs. 39.1%). Authors concluded that brimonidine was superior to timolol in visual field protection through a mechanism other than IOP reduction, proposing that this may be due to neuroprotective effect.[16]

In a recently our experimental study, we investigated effects of brimonidine on microglia cell morphology and compared with sham group. In the study, it was aimed to evaluate whether brimonidine has suppressive effect on neuroinflammation besides neuroprotective effects. In the study, it was found that the number of activated microglial cells was decreased when compared to the control group, suggesting that brimonidine may have beneficial effects on neuroinflammation.[17]

ROCK inhibitors are another remarkable group in recent years. The Rho proteins are small G proteins, which play role in cell proliferation, adhesion, migration and contraction. In addition, they have important roles in protection of cytoskeleton, gene expression and regulation of apoptosis. The ROCK is a serine-threonine kinase and has two effector isoforms (ROCK 1 and 2). It is a GTP-mediated protein. In a study on patients with POAG, a significant increase was observed in RhoA protein level. This may be due to excessive activation in the ROCK pathway. The ROCK inhibitors increase humor aqeuous efflux due to relaxation in ciliary muscle, trabecular network and cellular and morphological changes in Schlemm canal, reducing IOP.[18-20] In addition, they have antiscar effects. In addition, they enhance ocular blood flow through relaxation of vascular smooth muscles.[21]

In recent years, in glaucoma management, the finding that ROCK inhibitor may have potential protective effects has emerged another important, exciting activity of ROCK inhibitors. Although the mechanism hasn't been fully elucidated, it is thought that the effect may be directly due to ROCK inhibition. In addition, it has been reported that **Ripasudil**, a member of ROCK inhibitors, can prolong RGC survival by suppressing oxidative stress in pathways involving Nox1 family.[22] In another study, it was shown that topical Ripasudil in two different doses provided IOP reduction and decreased glaucomatous retinal degeneration.[23] In our previous study, we administered two different doses of Ripasudil (20µM and 50µM) in a model of mechanical optic nerve damage via intravitreal route and investigated the effects of Ripasudil on RGC survival and glial cell activation using immunohistochemical methods. In the study, it was found that both doses caused RGC loss and glial cell activation and provided significantly higher neuroprotection when compared to sham group.[24] **Netarsudil**, another member of ROCK inhibitors, has an inhibitor effect on norepinephrine transport, reducing episcleral venous pressure. In a experimental model, it was shown that netarsudil provides axonal protection.[25]

It was shown that the Coenzyme Q and Nicotinamide are molecules play active role in mitochondrial energy production in biochemical manner. The **Coenzyme Q** is an essential cofactor in electron transport chain in mitochondria, supporting ATP synthesis. However, it is a potent antioxidant and scavenges free radicals. In experimental models of glaucoma, RGC protective effects were shown for Coenzyme Q. In different studies were demonstrate, it can contribute neuroprotection via several mechanisms. These mechanisms include antioxidant activity and prevention of oxidative stress, antiapoptotic gene expression (Bad/Bcl-2 stabilization), anticytokine activity (decreased TNF-α release) and excitotoxicity blockage. Thus, it may play an effective role in the treatment of mitochondrial dysfunction.[26-28] Idebenon is an antioxidant with characteristics similar to Coenzyme Q and increases ATP production.[29] It can reach high concentrations in vitreous by topical administration with vitamin E (α Tocopherol). In our previous study, topical Coenzyme Q+α Tocopherol were compared to a sham group in the experimental model of optic nerve injury using immunohistochemical methods, it showing marked neuroprotective effects.[30] In addition, in a human study, Parisi et al. assigned POAG patients receiving ß blocker therapy into two groups and topical Coenzyme Q was given to a group over 12 months. Authors assessed electrophysiological test (PERG, VEP) results to investigate

retinal and cortical responses. In comparison at month 6 and 12, it was found that recordings were markedly better in Coenzyme Q group when compared to baseline.[31]

Nicotinamide (amid form of vitamin B3) is another remarkable molecule involved in mitochondrial energy production. The NAD is used in several metabolic pathways including glycolysis, fatty acid oxidation, tricarboxylic acid cycle and oxidative phosphorylation and has an important role in cellular ATP production. In addition, two major enzyme system consume NAD. These are PARPs responsible from DNA repair and SIRTs that play role regulation energy metabolism, gene expression, stress response and apoptosis. The NAD content is reduced by both aging and extensive consumption in the organism. In the context of neurodegenerative diseases, it seems that NAD metabolism is strongly associated to pathophysiology of axonal degeneration.[32] Thus, it was investigated whether NAM supplementation will be beneficial in glaucoma. In an experimental study, Williams et al. proposed that NAM can be beneficial in the prevention and treatment of glaucoma. Mitochondria can be more susceptible to damage in advanced age and elevated IOP, because NAM is decreased. It was found that NAM supplementation was successful in the protection of RGCs.[33] In addition, NAD also has vasoprotective effects, emphasizing hat it may contribute to endothelial functions. In oral therapy, dose can be safely to given up to 3 g/day. It is generally well-tolerated, adverse effects include flushing in the skin, bowel irritation, hyperglycemia and gout activation.[33] It is recommended to be cautious for hepatotoxicity, but its seen in extremely high doses. The supplementation can be given in NR formulation which has higher bioavailability. In a study, Hui et al. assigned 57 glaucoma patients receiving topical hypotensive into two groups to receive placebo or NAM (1.5 g/day during first 6 weeks and 3 g/day for additional 6 weeks. At the end of follow-up, visual functions are assessed using ERG and perimetry. It was found that inner retina functions were better (as assessed by ERG and perimetry) in the NAM group.[34] Definitely, additional studies are needed to evaluate long term effects. Moreover, previous studies showed that Nmnat isoforms (Nmnat 1, 2, 3) had protective role against axonal degeneration. It was reported that increased cytoplasmic Nmnat expression provided axonal protection in RGC in the glaucoma model.[35, 36] It is possible to obtain NAD by enhancing Nmnat 1 expression via gene therapy.[32]

Pyruvate is another molecule to increase energy in neuronal cells. It is derived from glycolysis, which can be converted to acetyl CoA (Krebs cycle) or lactate. Substantial amounts of ATP is supplied to cells by these biochemical processes. Oral pyruvate supplementation recovers hypometabolism, enhancing bioenergetic capacity of mitochondria and increasing ATP production. In addition, it potentiates antioxidant barrier. In an experimental model, it was shown that pyruvate provides neuroprotection by proposed mechanisms.[37] In a human study designed based on above-mentioned activities, significant recovery was achieved in visual field parameters of glaucoma patients in short term follow up by pyruvate (1.5-3 g) plus NAM (1-3 g) combination.[38] These two agents naturally occurs in the organisms and no severe adverse effect has been observed. Of course, the short term neuroprotective benefits should be tested in long-term studies.

Citicoline is another molecule predicted to have neuroprotective activity through multiple mechanisms. It contains ribose, cytosine and choline. It is a naturally occurring molecule approved by FDA. It is used in various clinical presentations such as CNS-related trauma, ischemia, dementia and neurodegenerative disorders, hyperactive/inattention, amblyopia and glaucoma. The mechanisms of action include preservation of mitochondrial membrane and its functions by cardiolipin synthesis, supporting cell membrane and its functions by phosphatidylcholine synthesis, enhanced axonal protection by myelin and sphingomyelin production, aiding axonal transport by acetylcholine, dopamine and serotonin production, potent antioxidant activity by increased glutathione production, prevention of glutamate related excitotoxicity, positive effects on endothelial function by NO modulation and endothelial Ca^{+2} release and increased Bcl-2 antiapoptotic gene expression.[39-42] It was shown that the citicoline provided protection in RGC and that it achieved effective concentration in vitreous via oral and topical route.[43, 44] In the work using electrophysiological tests, Parisi reported significant improvement in PERG and VEP responses in glaucoma cases receiving citicoline.[42, 43] In a study, Ottobelli et al. investigated use of 500 mg/day oral citicoline solution for 4 months; followed by 2-months drug-free intervals and reported slowed glaucoma progression in citicoline group during 2-years follow-up.[45]

Magnesium, an important mineral for organisms, can have beneficial effect regarding neuroprotection. It enhances ocular blood flow by regulation endothelial functions through ET1 regulation and NO modulation. In addition, it blocks NMDA receptor-related intracellular Ca^{+2} influx. It has a significant inhibitor effect on excitotoxicity pathway. It has minimal adverse effects via oral route and is well-tolerated.[46]

Ocular Surface Diseases in Glaucoma

In glaucoma cases, OSD are another important problem which affect treatment success. OSD are seen by 6% in individuals aged >40 years; which increases up to 15% in individuals aged>65 years. The OSD incidence is 40-

60% in glaucoma; 15-25% of which are severe cases. The importance of solution of the problem will be better understood given that the incidences of both diseases are increased by advancing age. The factors leading OSD may include dry eye, adverse effects of antiglaucomatous agents, and preservative agents in topical drugs.[47, 48] In addition, epithelial and endothelial dysfunction due to pseudoexfoliation, that is seen frequently in our country, can potentiate this problem.

ß-blockers can lead decreased amount of tear, corneal hypoesthesia and punctate epithelial keratitis. Dorzolamide can cause increase in corneal thickness, endothelial toxicity and allergic reactions while prostaglandin analogs may lead conjunctival hyperemia, punctate epitheliopathy, meibomian gland dysfunction and recurrent herpes. Brimonidine may be associated with follicular or allergic conjunctivitis. In addition, preservatives in topical preparations are also important. BAK is a cationic detergent, which is most commonly used preservative agent. It blocks drug contamination. It impairs lipid structure of cell membrane, increasing permeability. Although its beneficial in preventing contamination, this agent are highly toxic for ocular surface. The clinic picture will be worsened when a patient using glaucoma medication containing BAK are also prescribed artificial tear drops containing preservative agent (Figure 3). The toxic state will become more prominent. Although Poliquad (polyquaternium), Purite (stabilized oxychloro complex (95% chloride, 0.5% chlorate and Sofzia (sorbitol, borate, zinc) are less toxic preservative agents, their adverse effect on ocular surface cannot be negligible.[48] Prolonged glaucoma treatment, particularly with multiple agents, induce significant damage on ocular surface. An inflammatory state develops with pro-inflammatory cytokine release. Fibroblastic activity is increased in conjunctiva and tenon.[47, 48] As a result, when require a surgical intervention following medical treatment, may be high risk failure due to fibrosis development.

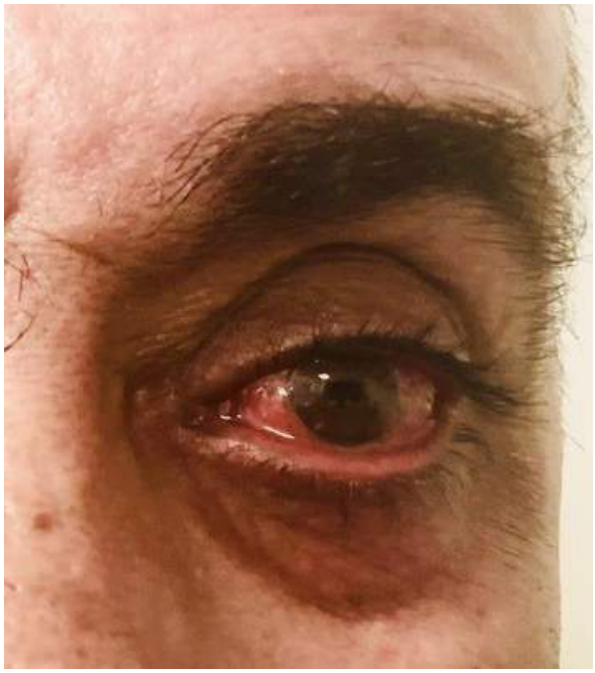

Figure 3: *Multiple antiglaucomatous agents and artificial tear with preservatives worsen clinical picture due to increased preservative burden.*

Thus, diagnosis and supportive care of OSD is an important component of management in glaucoma cases.[49] Firstly, ocular surface and functions of tear should be evaluated (OSDI scoring, tear break up time, Schirmer test, corneal staining pattern) and severity of OSD should be determined (Figure 4). Blepharitis should be treated if present (eyelid hygiene, topical and/or systemic antibiotics). In mild cases, preservative-free artificial tear drop can be given while topical cyclosporine in advanced cases; systemic agents should be added in refractory cases. Reduction of preservative burden is one of the key elements in the treatment plan.[50] It may be considered preferring fixed combinations instead multiple topical medications, using preservative-free agents as possible and finally proceeding with surgery rather prolonged use of >3 topical preparations in patients requiring treatment with multiple agents (Figure 5).

In cases with previous history of glaucoma surgery, ocular surface should be assessed meticulously. Bleb dysesthesia can be seen in some cases, particularly in those with history of filtration surgery. This has significant effect on patient comfort. In particular, increased bleb height (balloon bleb), multi-cystic blebs (Figure 6), those not covered by eyelid sufficiently and those with nasal localization may be problematic.[51] Development of dellen associated with bleb

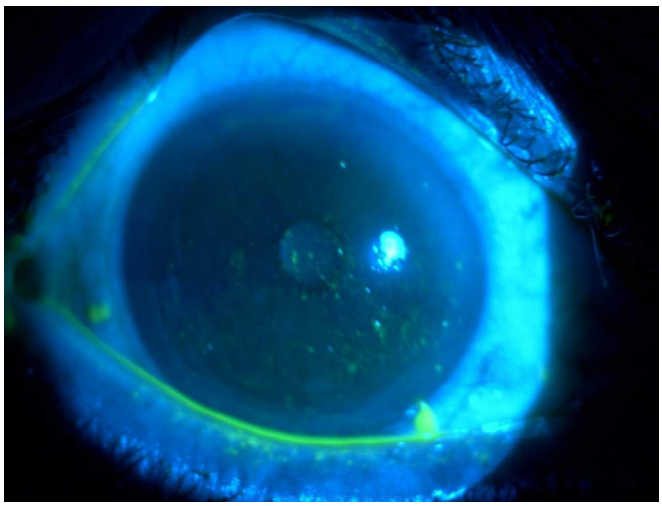

Figure 4: *Punctate corneal staining with fluorescein due to ocular surface disease in glaucoma patient.*

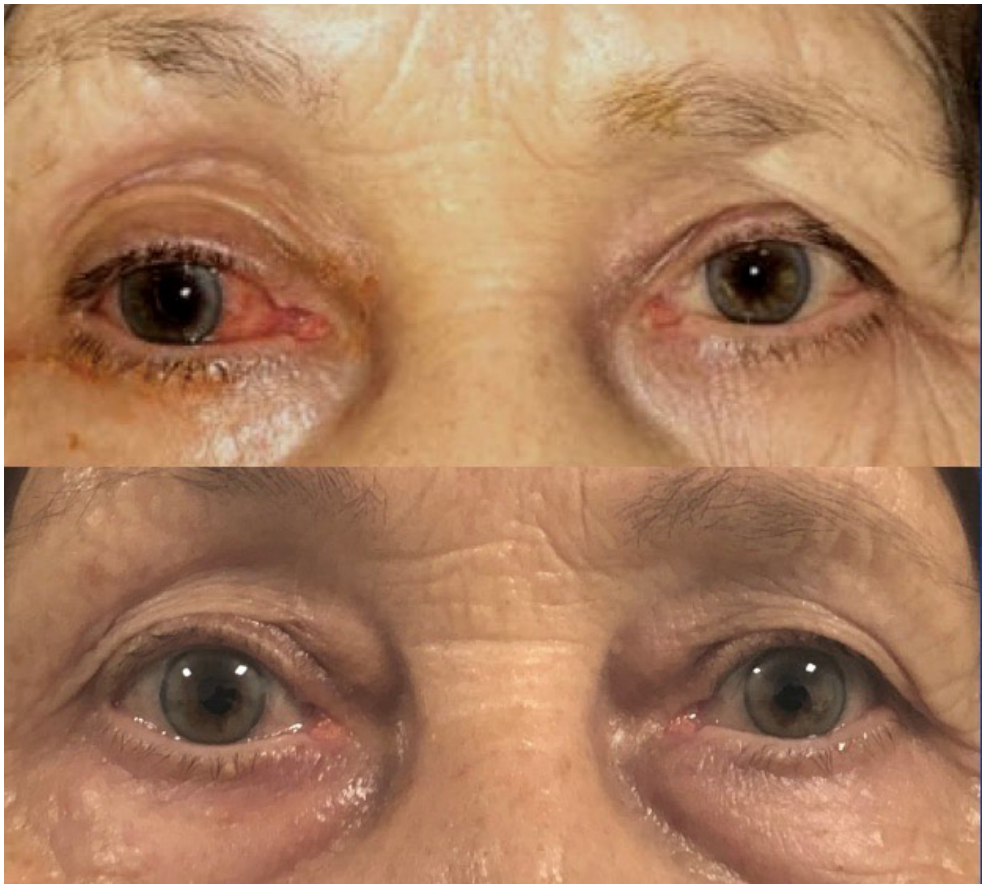

Figure 5: *A patient recommended to use multiple antiglaucmatous agent due to pseudoexfoliation glaucoma in right eye. The patient had compliance issues and IOP could not be controlled. Image on month 3 after filtration surgery following treatment of ocular surface disease.*

on corneal surface, epithelial problems and thin-walled blebs should be taken into account (Figure 7).

In addition to above-mentioned measures, the concept of nutritional support in OSD has gained increasing interest, becoming a part of treatment. We will discuss remarkable supportive molecules for ocular surface in this section of manuscript.

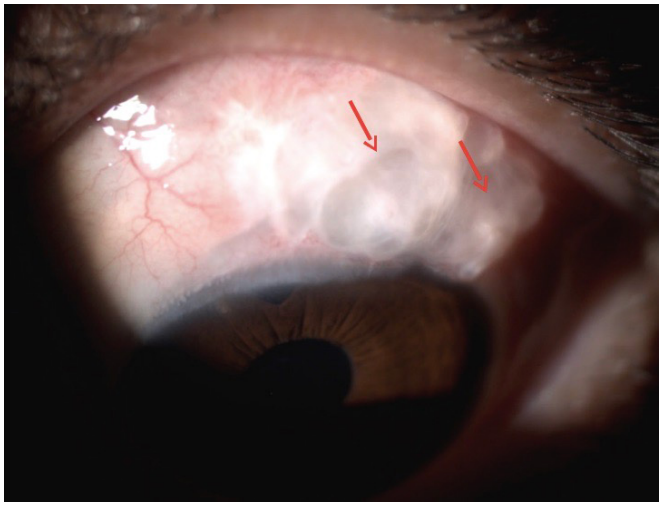

Figure 6: *Multi-cystic bleb.*

Currently Remarkable Molecules on the Ocular Surface

Curcumin is a polyphenol isolated from curcuma longa. It is also known as turmeric. In studies, it was shown that curcumin has potent antioxidant activity and leads significant increase in antioxidant enzyme activity including catalase and glutathione peroxidase. It also exerts potent antiinflammatory (decrease in cyclooxygenase, lipoxygenase and leukotrienes) and anticytokine effects (decrease in IL-1, IL-6, IL-8 and TNF-α secretion). It reduces proapoptotic gene expression and increases antiapoptotic gene expression. The curcumin supports neurotrophic factor release. It also supports corneal homeostasis through inhibition of FGF and VGEF release; in addition, it suppresses neovascularization.[52-54] Thus, it seems to have positive effects regarding RGCs, ocular surface protection and homeostasis. In bioavailability studies, it was found that bioavailability was higher in tetrahydrocurcumin form.[52] However, curcumin in

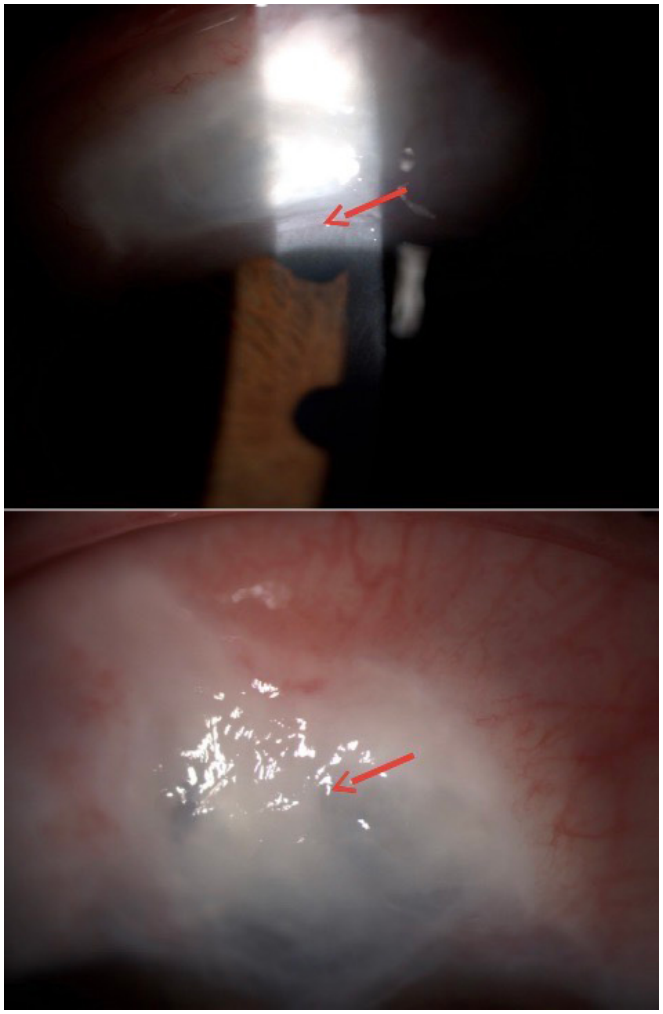

Figure 7: *Development of dellen associated with bleb (upper), thin-walled bleb (bottom).*

combination with piperine (found in black pepper) can improve absorption by 2000%.

Resveratrol is a naturally occurring polyphenol from non-flavonoid stilbene family. In plants, it is synthesized in response to infection, mechanical damage and UV radiation. It has two isometric forms (cis and trans). The trans form is light-sensitive, active form. It is found in grape skin and seed, blueberry, bitter chocolate and peanut. It is a hydrophobic molecule. Studies showed that, similar to curcumin, resveratrol has potent antioxidant, antiinflammatory and antiapoptotic effects.[52,53] It was reported that resveratrol protected trabecular cells against apoptosis in glaucoma model.[57, 58] Resveratrol can also induce sirtuins strongly.[53]

It may have beneficial effects in neuronal protection and supporting ocular surface. Bioavailability is highly limited due to molecular structure and poor solubility when given via oral route (Resveratrol paradox). Thus, it is important to achieve concentration that may show beneficial effects in supportive therapy. Significant advances have been achieved to improve bioavailability using transport systems (nanoparticle, liposomal transport systems, SLN). It is though that sustained release can prevent rapid metabolism and excretion of the molecule.[53]

However, transportation with nanoparticles can be pioneer in development of formulations compatible with topical ocular use too for curcumine and resveratrol.[59]

Omega 3 is another important molecule for ocular surface. It has been reported that omega 3 has beneficial effects on ocular surface such as decreased corneal inflammation, improvement in Meibomian gland functions and increased tear production. Omega 3 supplementation alleviates dry eye symptoms and ocular surface problems while improves Meibomian gland dysfunction. The increased omega 6 in diet (impaired omega 6: omega 3 balance in favor of omega 6; which should be 4:1 but 15: 1 in Western diet) leads negative effects on tear film quality and ocular surface. Thus, omega 3 supplementation may have significant contribution to protection of ocular surface.[55,56]

Antioxidant molecules vitamin C and E has remarkable effects on ocular surface. **Vitamin C** enhances antioxidant capacity of ocular surface and supports collagen synthesis. It has important role in corneal wound healing. In addition, in combination with **Vitamin E**, it leads increased tear film production and stabilization.[60] **Vitamin D** exerts immunomodulatory effect by regulating T lymphocyte response. It improves barrier function of corneal epithelium with strong support to tear film production and stability. **Vitamin B12** plays an important role in myelin synthesis. It is recommended in many neuropathic condition. In recent years, the role of neurosensory abnormality has been better understood in the pathophysiology dry eye. Topical and parenteral vitamin B12 supplementation is associated with symptomatic recovery in cases with dry eye. Thus, it is thought that it can have a role in neurosensory component of OSD and supplementation with vitamin B12 may be beneficial in recovery.[54, 55]

When a molecule is considered as a supplement in the light of above-mentioned mechanisms and studies, pharmaceutical form and dose should be appropriate. At this point, it becomes important to audit formulations manufactured and trust in manufacturers. In addition, interventions that may improve bioavailability (more effective formulations, combinations and drug delivery systems) should also be taken into consideration. Another important issue is cost-effective in the presence of current economic issues. Definitely, cost-effectiveness analysis are needed to support use in clinical practice.

Given these considerations, it is apparent that there is a need for road map focusing on three aspect in glaucoma therapy.

IOP control, prevention of neuronal damage and ocular surface disorders should be addressed together to ensure treatment success. We will have novel treatment options by improving understanding regarding mechanisms of cellular damage (neurodegradation and neuroinflammation). Thus, treatment strategies will be updated by introduction of novel molecules. In particular, studies on neurobiology and nano technology will be pioneering.

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