·Commentary·

New ideas for medical therapy of glaucoma in the future

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Abstract

• New drugs are developed rapidly with novel ideas of action mechanisms for the treatment of glaucoma. The most classic drugs under development are to lower the intraocular pressure (IOP). New agents were invented to lower the IOP through ① induction of metalloproteinases (MMPs), ② contraction of trabecular meshwork cells, ③ inhibition of aqueous humor secretion, and ④ activation of CB-1 receptor, *etc.* The second class of drugs under development is intended to improve the ocular blood flow (OBF), particularly in retina and optic nerve head (ONH). Drugs that improve the OBF irrespective of the IOP changes could be quite useful for the treatment of normal tension or low-tension glaucoma. Neuroprotection is the latest developed mechanisms of glaucoma treatment. Although the history of neuroprotection research is very short, there are many agents under investigation in this class. They include ① blockade of N-methyl-D-aspartate receptor, ② neurotrophic agents, ③ inhibition of inducible nitric oxide synthases (iNOS), ④ inhibition of apoptosis, ⑤ protective autoimmunity, ⑥ stem cell therapy, and so on. Since all drugs for glaucoma treatment are used to stabilize the disease rather than to cure it, it is critical that an ideal drug with high therapeutic index and low cost price should be invented.

• KEYWORDS: glaucoma; treatment; intraocular pressure; ocular blood flow; neuroprotection

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INTRODUCTION

Numerous novel ideas are evolving lately for the medical treatment of glaucoma. Despite continued advances in laser and incisional surgery, pharmacological therapy still plays the most important role in glaucoma therapy. Although various agents and mechanisms are under investigation, the endpoints are still related to the reduction of intraocular pressure (IOP), improvement of ocular blood flow (OBF), and/or protection of neuron function.

TO LOWER IOP

IOP is an Important Factor in Glaucoma Elevated intraocular pressure (IOP) is one of the major risk factors for glaucoma and reducing the IOP remains the mainstream of glaucoma therapy. Good evidence from multiple large randomized trials shows that reducing IOP is effective in preventing disease progression in ocular hypertension in primary open angle glaucoma, and even in so called normal tension glaucoma[1-3].

Current Medical Treatment Medical treatments available today include sympatholytic agents (timolol,carteolol, betaxolol, levobunolol, *etc.*), prostaglandin analogues (latanoprost, travoprost, bimatoprost), parasympathomimetic drugs (pilocarpine), sympathomimetic drugs (brimonidine, apraclonidine) and carbonic anhydrase inhibitors (topical: dorzolamide, brinzolamide; oral: acetazolamide) [4]. They lower IOP mainly by reduction of aqueous production or increase of aqueous outflow[4-8].

New Drug Development –to Lower IOP through Increase of Aqueous Outflow Glaucoma is often characterized by decreased pressure-sensitive aqueous outflow through the trabecular meshwork. The impairment in outflow facility causes the high intraocular pressure and large IOP fluctuations often found in glaucoma. Thus, outflow facility is an attractive target for glaucoma treatment because enhancing outflow facility tends to restore the self-regulating tendency of IOP[9].

Future intraocular pressure-lowering therapies for glaucoma may better be directed at enhancing aqueous outflow through trabecular meshwork. Kiland *et al* [10] found that in cynomolgus monkeys, decreasing aqueous humor formation with timolol and dorzolamide over a period of time could lead to reduction in outflow facility, particularly when the therapy was combined with agents that redirect aqueous from the trabecular meshwork, such as prostaglandin F2 analogues. That indicated long-term use of drugs that

suppress aqueous humor formation, or drugs that redirect aqueous humor outflow from the trabecular meshwork could cause underperfusion of the trabecular meshwork and a secondary decrease in outflow facility. Although there was also evidence suggesting that long-term therapy with bimatoprost increased both pressure-dependent trabecular outflow and pressure-independent uveoscleral outflow via remodeling of extracellular matrix in the trabecular meshwork and ciliary muscle, respectively[11-14].

The trabecular meshwork forms most of the resistance to aqueous humor outflow needed for maintenance of a pressure gradient between intraocular pressure of approximately 17mmHg venous pressure of approximately 10mmHg [15,16]. The trabecular meshwork may be considered the extracellular matrix (ECM) of the trabecular cells. This is a network of elastic fibers that is embedded in collagenous tissue forming an intertrabecular space filled by mucous substances. The large fibrillar collagens provide the basic structural framework, and the microfibrillar and globular collagens, with the proteoglycans and glycosaminoglycans, fill the interstices of the large molecule [16,17]. Cavallotti *et al*[17] demonstrated the major age-related changes of the trabecular meshwork were an increase in extracellular material and increase in its electron density. The most abundant extracellular material in young eyes is a fine granular or fibril material. However, with increasing age, the fibril-granular material decreases, while an electron-dense material becomes prominent. In fact, in young subjects, the glycosaminoglycans are prevalently of low molecular weight, while in old objects, those are of high molecular weight. Li *et al* [18] reported excessive synthesis of an ECM component, fibronectin, underlying monolayer of human trabecular meshwork cells, and reduced monolayer permeability. Excessive, abnormal accumulations of ECM materials have been noted in the trabecular meshwork of eyes obtained from patients with primary open angle glaucoma [15,16]. That was supposed to be the reason of increased resistance of aqueous outflow.

Baseline degradation and synthesis is omnipresent although ECM appears to be a static component. Many enzymes from several different classes of molecules are involved in the process of matrix degradation. And it can be divided into 2 categories: the extracellular secretion of catabolic enzymes and the internalization of molecules with subsequent lysosomal degradation. The most important of the secreted enzymes, which are responsible for the bulk of matrix degradation, are the matrix metalloproteinases (MMPs). These proteins have been grouped in the same family on the basis of extracellular matrix specificity, zinc dependence, sequence homology, secretion as a proenzyme, and inhibition by another class of proteins, the tissue inhibitors of metalloproteinases (TIMPs). Another common family of secreted enzymes is the serine proteinases. Examples of these enzymes are the tissue-type plasminogen activator (TPA) and the urokinase-type plasminogen activator (UPA). Several lines of evidence supported the notion that the serine proteinases were involved with the MMPs in an ECM degradation cascade and that the serine proteinases were upstream in this hierarchy[19].

Recent evidence has suggested that MMPs play a role in numerous modern glaucoma therapies, including topical prostaglandin analogues, topical steroids, and argon laser trabeculoplasty. Additionally, direct and indirect regulation of this system has been shown to increase aqueous humor outflow facility. It is possible that therapies directing at modulating specific enzymes represent the next generation of glaucoma therapy[19].

In addition, the aqueous humor pathways through the subendothelial layer can be influenced by ciliary muscle contraction and presumably also by contractile elements recently found both in trabecular meshwork and scleral spur. Pharmacologically induced disconnection of cribriform cells and inner wall leads to wash out of extracellular material through breaks of the endothelial lining of Schlemm's canal and to increase of outflow facility[15].

Possible Development of Drugs

Increase outflow by MMPs inductors Matrix metallo-proteinases (MMPs) are involved in trabecular meshwork extracellular matrix metabolism and have been shown to increase aqueous outflow facility.

Pang *et al*[20] reported his study on 5 human TM cell strains. All of them produced detectable basal amounts of proMMPs and TIMPs. Interleukin-1 alpha was most efficacious in increasing proMMP-3 production with an EC(50) of 0.5μg/L. The IL-1alpha-induced upregulation of proMMP-3 correlated well with an increase in MMP-3 activity.

Another report from Pang showed activator protein (AP-1) stimulators, such as beta-naphthoflavone, 3-methylcholan-threne, and tert-butylhydroquinone (tBHQ), significantly upregulated (2-4-fold) trabecular meshwork cell expression of MMP-3. The stimulatory effect of tBHQ was concentration dependent, with an EC (50) of approximately 3μmoL/L, and was blocked by concomitant treatment with 100nmoL/L SR11302, which sequesters AP-1. When nonglaucomatous human eyes were perfused with tBHQ (10μmol/L), both outflow rates and perfusate proMMP-3 level increased significantly within the first 24 hours. The outflow effect of tBHQ was suppressed when SR11302 (100nmoL/L) was added in the perfusate. The IOP was also lowered with tBHQ by more than 40% in perfused glaucomatous eyes. So an AP-1 activator, tBHQ, upregulated the expression of MMP-3 in cultured human trabecular meshwork cells and perfused

human eyes, and enhanced outflow *ex vivo*. These effects were blocked by sequestering AP-1, suggesting that activation of AP-1 can lead to increased MMP-3 production in the trabecular meshwork , which in turn improves outflow facility[21].

Serial studies of Pang and his colleagues indicated that activation of protein kinase C micro (PKC micro), mitogen-activated protein kinase kinase (MEK), and p38 leading to the activation of AP-1 was critical to the IL-1alpha-stimulated upregulation of MMP-3 in human trabecular meshwork cells. Therefore, it was likely that compounds that activated the AP-1 pathway would upregulate the production of MMP-3 and improve aqueous outflow [22]. This unique mechanism may provide a novel therapy for glaucoma.

Increase outflow by H -7 The serine-threonine kinase inhibitor H-7 (1- [5-isoquinoline sulfonyl]- 2-methyl piperazine) inhibits actomyosin-driven contractility, probably by inhibiting myosin light chain kinase or rho kinase. This leads to deterioration of the actin microfilament system and perturbation of its membrane anchorage and to loss of stress fibers and focal contacts in many types of cultured cells, including human trabecular cells. H-7 administered intracamerally or topically to living cynomolgus monkeys increases outflow facility and decreases IOP by a mechanism independent of the ciliary muscle, presumably acting directly on the trabecular meshwork. A morphological study of the TM in the live monkey eye indicated that H-7 reduced trabecular meshwork cell contractility, thereby "relaxing" the trabecular outflow pathway, expanding the draining surface, and facilitating flow through the meshwork. Because the effect of H-7 on outflow resistance is pressure dependent, it is assumed that H-7 may be more effective in the glaucomatous eye with elevated IOP. Twenty micro liter of 400mmoL/L H-7 (approximately 15%) decreased IOP in living monkeys when administered topically, but also produced transient changes in corneal endothelial cells and corneal thickness when applied to the central cornea as 4 drops of 5-μL volume. In normotensive eyes, one dose of 5% H-7 maximally decreased IOP by a mean±SEM of 2.5× 1.0mmHg (-16.7%×5.5%) at 3 hours. In glaucomatous eyes, one dose of 2% H-7 maximally decreased IOP by a mean× SEM of 5.8×0.6mmHg (-16.9%×1.6%) at 2 hours. Five percent H-7 increased outflow facility and decreased IOP, but did not affect corneal thickness. Multiple doses of H-7 induced greater reduction of IOP than a single dose. As assessed by slitlamp biomicroscopy, 5% H-7 was also less toxic to the corneal epithelium[23].

Decrease aqueous inflow with A3 receptor antagonists Aqueous humor is secreted across the ciliary epithelium by transferring solute, chiefly NaCl, from the stroma to the posterior chamber of the eye, with water passively following. The epithelium consists of two layers: the pigmented ciliary epithelial (PCE) cells abutting the stroma, and the non-pigmented ciliary epithelial (NPCE) cells facing the aqueous humor. Gap junctions link adjacent cells within and between these layers. Secretion proceeds in 3 steps: ①uptake of NaCl from stroma to PCE cells by electroneutral transporters; ② passage of NaCl from PCE to NPE cells through gap junctions, and ③ release of Na^+ and Cl^- through Na^+, K^+-activated ATPase and Cl^- channels, respectively[24].

Adenosine stimulates Cl^- transport by NPCE cells. Civan and co-workers studied on all four known adenosine receptor subtypes, and found immortalized human NPE cells and rabbit ciliary processes possess A3 receptors. It was by stimulating these A3 receptors that adenosine can activate Cl^- channels in NPE cells [25]. In comparison of A3 receptor knockout mice (A3R-/-) with control mice (A3R+/+), IOP was significantly lower in A3R knockout mice than in normal mice. And the selective A3 antagonists MRS1191 lowered IOP in the control mouse [26]. Okamura *et al* [27] reported a study on structure-activity relationships of adenosine A3 receptor ligands. The potent and selective A3 receptor antagonists were identified and evaluated in a monkey model of intraocular pressure by eye-drop administration. And compound OT-7999 was found to significantly decrease IOP in the animal model. These suggest that reducing Cl-channel activity with A3 antagonists may provide a novel approach for treating glaucoma.

Activation of CB1 by cannabinoids A 25% -30% IOP lowering effect of smoking marijuana was reported early in 1971. Since then, numerous studies have been conducted confirming that different cannabinoids, including cannabidiol, cannabigerol, endogenous cannabinoids, and some synthetic cannabigerols, can reduce the IOP when administered systemically and topically. The mechanism of action of cannabinoids in the human eye was not fully understood. It was found that both A3 and CB1 receptors activated a PKC-sensitive Cl^- current in human NPCE cells[28]. Pharmacological and histological studies supported the direct role of ocular CB1 receptors in the IOP reduction induced by cannabinoids. CB1 receptors were detected in ocular tissues of the human eye, including the ciliary epithelium, the trabecular meshwork, Schlemn's canal, ciliary muscle, ciliary body vessels and retina. And high levels of CB1 mRNA in the ciliary body were found. The anatomical distribution of cannabinoids was shown on trabecular and uveoscleral aqueous humor outflow and on aqueous humor production. Cannabinoids have the potential of becoming a useful treatment for glaucoma. However, several challenges need to be overcome, including unwanted systemic side effects (psychotropic, reduction in systemic blood pressure),

possible tolerance, and the difficulty in formulating a stable and effective topical preparation [29].

TO IMPROVE OCULAR BLOOD FLOW

It is well known that high IOP is not necessary responsible for the development of all glaucoma cases and that reducing IOP is not necessary effective in avoiding the progression of each and every glaucoma patient. Collaborative Normal-Tension Glaucoma Study Group found 20% of normal tension glaucoma patients achieving 30% reduction in IOP were estimated to progress after 5 years [2]. Fourteen percent of the patients with 100% of IOP measurements below 18mmHg were estimated to progress after 7 years in the Advanced Glaucoma Interventional Study [1]. The Early Manifest Glaucoma Trial randomized patients with early glaucomatous damage to treatment *vs* no treatment. Although the standard treatment reduced the risk of progression by 50%, 45% of the treated patients still showed progression after a median follow-up period of 6 years [30,31]. So IOP reduction is not 100% effective in preventing the progression of glaucomatous optic nerve damage. Glaucoma is a multifactorial condition and there are other factors participating in the pathogenesis of the disease.

Ocular blood flow (OBF) disturbance is one of the possible factors. A number of systemic vascular disorders have been associated with glaucoma, including diabetes, hypertension, peripheral vascular disease, and migraine. Different techniques demonstrated a reduced ocular perfusion in glaucoma patients at the optic nerve head (ONH), choroid, retina and retrobulbar vessels [32,33]. Experimental studies demonstrated that axonal loss and demyelination of the prelaminar portion of the optic nerve could be produced in animals by injecting endothelin-1, a vasoactive substance, with no change in IOP [34,35]. A number of mechanisms have been suggested to explain why OBF reduction may lead to glaucoma, including increased resistivity to flow, reduced perfusion pressure, and impaired autoregulation. Increased resistivity could be caused by anatomical variations, vasculitis, arteriosclerosis, or vasospasm. Reduced perfusion pressure might be secondary to an increased IOP or decreased blood pressure. Autoregulation deficits, characterized by the inability to compensate for changes in IOP or blood pressure in order to maintain adequate perfusion have been described in the retina; and ONH circulation in both experimental and glaucoma patients[32,33].

It is interesting to note that ample data are available on effects of antiglaucoma drugs and systemic medications on the various ocular beds.

Some antiglaucoma medications have the potential to directly improve ocular blood flow[33]. Unoprostone appears to have a reproducible antiendothelin-1 effect. Unoprostone isopropyl is a derivative of a prostaglandin metabolite. In one experiment employing the hydrogen gas clearance flowmeter, the effect of endothelin-1, applied to the rabbit eye in order to cause vasoconstriction, was significantly reduced at 2.5 and 3.5 hours after the intravitreal injection of 10μL of unoprostone 0.06% with no significant reduction in IOP [36]. In a placebo-controlled, randomized and double-masked study, choroidal blood flow and fundus pulsation amplitude were decreased after administration of exogenous ET-1 in 24 healthy individuals. This effect was significantly blunted when topical unoprostone was coadministered. The authors demonstrated a functional antagonism between ET-1 and topical unoprostone in the choroidal vasculature [37]. However, significant improvement of POBF or ocular hemodynamics was not found in normal volunteers and normal tension glaucoma patients [38, 39]. While there were evidence showing circulation on optic nerve head was increased[40,41].

Betaxolol may exert a calcium-channel blocker action and improve OBF by avoiding non-selective beta-blocker-induced vasoconstriction. In experimental studies, topical betaxolol was found to increase tissue blood flow in rabbits. Although the acute effects of betaxolol instillation in healthy humans or glaucoma patients tended to show no significant change or even reduction in circulation, chronic use of betaxolol has been shown to improve retinal and ONH blood flow[33].

Carbonic anhydrase inhibitors seem to accelerate the retinal circulation. Several investigators have confirmed that acetazolamide is capable of increasing cerebral blood flow in a dose-dependent manner, which may, at least in part, be attributed to extracellular acidification. Evidence from ocular hypertensive rabbits and healthy volunteers supported the drug's ability to increase OBF[33].

Regarding the systemic drugs, calcium channel blockers apparently did not evoke a uniform response in glaucoma patients. Some showed an improvement of OBF, whereas others either did not show a change or developed deteriorations of hemodynamic parameters and visual function [33]. Luksch *et al* [42] reported single dose of nimodipine (60mg) could increase ocular blood flow significantly in NTG patients. Mean ocular fundus pulsation amplitude, measured with laser interferometry, increased by 14% (SD 14%) (P=0.0008). Optic nerve head blood flow and choroidal blood flow, assessed with laser Doppler flowmetry, increased by 18% (SD 16%) (P=0.0031), and 12% (SD 14%) (P<0.001) separately. Sixteen patients with NTG and clinical signs of vasospastic hyperreactivity, such as suffering from extremely cold hands and feet, were consecutively selected out of the local glaucoma registry in Michalk's study. Ten healthy age-matched volunteers were included as controls. Retinal capillary blood flow was

measured by Scanning Laser Doppler Flowmetry in both eyes before and 90±10 minutes after a single oral dose of 30mg nimodipine. Before administration of nimodipine, retinal capillary blood flow was significantly reduced in NTG patients compared with controls (262±80 *vs*487±164 AU, P<0.001). Nimodipine increased retinal capillary blood flow in NTG patients by 91±73% (P<0.001) to values of healthy controls (440±113 *vs*439±123 AU, P=0.635). In controls, nimodipine did not show significant effects. In NTG patients with additional vasospastic symptoms, retinal capillary blood was significantly reduced in comparison with healthy controls. Single-dose nimodipine yields to a normalization of retinal circulation in NTG patients up to values of healthy controls 90min after drug administration[43]. However, another investigation did not come to the same result. Thirty-two healthy subjects (21- 49 years old, mean age 28 years, 10 male, 22 female) received either nimodipine 30mg twice a day or a placebo according to the same dosage regimen in a double-blind cross-over study design. Ocular blood flow and optic nerve head blood flow were increased slightly but not significantly in the nimodipine group and remained unchanged or were even lower in the placebo group [44]. So, maybe the glaucoma patients, especially patients with a vasospastic response might get benefit from administration of nimodipine.

The evidence we have in the treatment of glaucoma does not support the use of other vasoactive substances, e.g. inhibitors of the renin-angiotensin system, Ginkgo biloba, magnesium, and dipyridamole. Longitudinal, prospective, randomized trials are needed[33].

NEUROPROTECTION

Studies have shown that regardless of the nature of neuronal injury, damage spreads beyond the directly injured neurons to affect adjacent neurons that escaped or were only partially affected by the primary lesion[45]. Progressive death of retinal ganglion cell (RGC) is the main characteristic of glaucomatous optic neuropathy[46]. Neuroprotection only interferes with secondary degenerative events, which focuses on minimizing the progress of glaucomatous neuropathy by interacting with neuronal processes[45].

Many compounds have been implicated as neurotoxic mediators of secondary degeneration. These include excitatory amino acids (e.g. glutamate), free radicals, nitric oxide, lipid peroxidation products (e.g. PUFAs), eicosanoids, cations, monoamines (e.g. 5-HT) and opioids. Accordingly, substances that prevent the formation or antagonize the action of these compounds have been investigated as potential neuroprotectants. Hundreds of agents have been tested in animals, but only a few have reached phase III clinical trials and only one, riluzole, has achieved FDA approval for clinical use (in amyotrophic lateral sclerosis)[45].

Different models have been used to study RGC damage, including crush/axotomy model, ischemia/reperfusion model, excitotoxic damage model, induction of elevated IOP model, *etc*.[46]. Among them, acute partial crush lesion of the rat optic nerve and animal model of glaucoma are most widely used.

NEUROPROTECTIVE COMPOUNDS

N-methyl-D-aspartate (NMDA)-receptor antagonists Studies have provided evidence of apoptosis associated with RGC death in human eyes with glaucoma [47]. Glutamate was shown to induce apoptosis in retinal cells as a mediator of an ischemic insult through hyperactivation of the NMDA receptor, and NMDA has shown a similar effect directly[45]. MK-801, a potent non-competitive NMDA-receptor blocker was found to triple the survival rate of neurons after partial crush injury of the rat optic nerve [48]. NMDA-receptor activity, however, is also essential for normal neuronal function. This means that potential neuroprotective agents that block virtually all NMDA-receptor activity will have unacceptable clinical side effects. Studies have shown that another antagonist, memantine, blocks only excessive NMDA-receptor activity without disrupting normal activity [49]. Memantine was shown to protect rat and rabbit retinal cells after ischemia/reperfusion injury and protect ganglion cells in a model of chronic elevation in vitreal glutamate. Hare and coworkers reported that histologic measurements of RGC survival, as well as recordings of the electroretinogram (ERG) and the visually-evoked cortical potential (VECP) showed that systemic treatment with memantine is both safe and effective to reduce structure changes and functional loss associated with experimental glaucoma in monkey [50,51]. Memantine was effective in clinical trials of a variety of dementia, mainly Alzheimer's disease, without causing the toxic side effect of MK-801. It is currently the only compound in phase III clinical trials for glaucoma[45].

Adrnergic agonists and antagonists The (2- adrnergic agonists, apraclonidine and brimonidine, were used in the treatment of glaucoma for lowering IOP. Several reports have linked these and similar agents with a neuroprotective role. A single injection of brimonidine resulted in a significantly smaller loss of RGCs and action potential amplitude after partial crush injury of the rat optic nerve. Pretreatment with brimonidine had a marked protective role on RGC loss, as evidenced by histology, cell labeling, and ERG. The possible mechanism may be attributed to preventing an increase in the vitreal content of glutamate and aspartate. Clinical trials are in progress to investigate the ability of brimonidine to protect human retinal ganglion cells and the visual field in glaucoma-related disease[45,52].

Betaxolol, a (1-adrenergic receptor blocker protected RGCs from ischemia/reperfusion insult in rat and rabbit eyes. The

neuroprotective effect of betaxolol was attributed to the drug's action as a calcium-channel blocker[45].

Calcium –Channel Blockers An increase in intracellular calcium exerts a neurotoxic effect through the activation of Ca^{2+}-dependent catabolic enzymes, leading to lethal alteration of the cell's metabolism. Flunarizine, a potent antagonist of the neuronal voltage-dependent calcium channel, was given daily to rats after unilateral axotomy of the optic nerve and found to somewhat enhance RGC survival 14 days after axotonomy. Both flunarizine and lomerizine were found to have a protective effect after induction of retinal ischemia in rats by elevated IOP [45]. It was found that nimodipine 30mg twice a day significantly increased in contrast sensitivity during treatment in healthy subjects, which did not correlate with an increase in ocular or optic nerve head blood flow[53]. According to the report of Michalk, in NTG patients with additional vasospastic symptoms, retinal capillary blood was significantly reduced in comparison with healthy controls. Single-dose nimodipine yielded to a normalization of retinal circulation in NTG patients up to values of healthy controls 90 minutes after drug administration[54]. A beneficial effect of treatment with calcium channel blockers was suggested by one moderate-term clinical analysis of patients with normal tension glaucoma[55].

Neurotrophic Factors Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor family of proteins, has been found highly effective in reducing the rate of RGC death after optic nerve crush in cats. While in glaucoma, death of RGCs may be partly due to a decrease in axonal transport of trophic factors [45]. Overexpression of the BDNF gene was achieved by intravitreous injection of a modified adenoassociated viral (AAV) vector capable of efficient transfection of retinal ganglion cells (RGCs). And it was found that BDNF could protect RGC as estimated by axon counts in a rat glaucoma model[56].

Rudzinski presented molecular, biochemical, and phenotypic evidence that significant neurotrophic changes occurred in retina, which correlated temporally with RGC death. After 7 days of ocular hypertension there was a transient up-regulation of retinal NGF, while its receptor TrkA was up-regulated in a sustained fashion in retinal neurons. After 28 days of ocular hypertension there was sustained up-regulation of retinal BDNF, but its receptor TrkB remained unchanged. Throughout, NT-3 levels remained unchanged but there was an early and sustained increase of its receptor TrkC in Müller cells but not in RGCs. These newly synthesized glial TrkC receptors were truncated, kinase-dead isoforms. Expression of retinal p75 also increased late at 28 days. Asymmetric up-regulation of neurotrophins and neurotrophin receptors may preclude efficient neurotrophic rescue of RGCs from apoptosis. A possible rationale for therapeutic intervention with Trk receptor agonists and p75 receptor antagonists was proposed [57].

Inducible Nitric Oxide Synthase Inhibitors Nitric oxide (NO) is an important biological mediator in the living organism. However, the overproduction of NO which is catalyzed by iNOS, a soluble enzyme and active in its dimeric form, is cytotoxic. Excessive nitric oxide, generated by inducible NOS-2 in astrocytes and microglia in the ONH of patients with glaucoma, may contribute to the optic neuropathy associated with the disease. A rat model of glaucoma, in which there is chronic, moderately elevated IOP and slow loss of RGCs, has been established to study pharmacological agents that have the potential to be neuroprotective. In this model, the pharmaco-logical use of an inhibitor of NOS-2, aminoguanidine, significantly prevented the loss of RGCs. A well-tolerated pharmacological inhibitor of NOS-2, perhaps orally or locally delivered, is a reasonable candidate for a neuroprotective agent for treating glaucoma[58].

Apoptotic cascade Another possible neuroprotective approach is interference with the final common pathway of apoptosis. RGCs have been shown to die by apoptosis, or programmed cell death. Central to apoptosis is the activation of specific proteases, termed caspases. Caspases are activated in chronic neurodegener- ations such as Alzheimer's disease (AD) as well as in RGCs after optic nerve transection. In rat glaucoma models, caspase-3, a major effector of the apoptotic cascade, was activated in RGCs and cleaved amyloid precursor protein (APP) to produce neurotoxic fragments that include amyloid-beta. Caspase-8, which initiated apoptosis after activation of receptors of the tumor necrosis factor (TNF) superfamily, was also activated in RGCs. This suggests a new hypothesis for RGC death in glaucoma involving chronic amyloid-beta neurotoxicity, mimicking AD at the molecular level. With loss of the protective effect of APP and upregulation of toxic APP fragments, RGCs die from chronic caspase activation, loss of synaptic homeostasis, amyloid-beta cytotoxicity and excitotoxicity. The benefits are that treatments for AD could be used to treat glaucoma, and strategies developed to treat glaucoma could treat other neurodegenerations [59]. For example, R(-)-1-(benzo[b] thiophen-5-yl)-2-[2-(N, N- diethy-lamino) ethoxyl hydrochloride (T-588) is developed to treat the dementia associated with AD and is now undergoing clinical trials. A recent study showed that T-588 had a neuroprotective effect against RGC death caused by elevated IOP and optic nerve crush in the rat[60].

Ginkgo biloba extract In Germany and France, Ginkgo biloba extract, obtained from green leaves of the G. biloba tree, is one of the most commonly prescribed drugs. It has been reported to prevent ischemic-induced oxidation, improve cerebral blood flow and antagonize the action of

platelet-activating factor. In a unilateral chronic moderately elevated IOP rat model, RGC loss in eyes with elevated IOP was 29.8%±1.5% at 5 months in untreated animals and 4.6% ±4.5% at 5 months in treated animals with Ginkgo biloba extract EGb 761. That supported that EGb 761 was an effective neuroprotectant in a rat model of chronic glaucoma[61].

To evaluate the effect of GBE on preexisting visual field damage in patients with normal tension glaucoma (NTG), 27 patients with bilateral visual field damage resulting from NTG received 40mg GBE, administered orally, three times daily for 4 weeks. With GBE treatment, a significant improvement in visual fields indices was recorded. The mean deviation (MD) at baseline was 11.40±3.27dB, while it was reduced to versus 8.78±2.56dB (t=8.86, P=0.0001, Chi-square test) after GBE treatment. Corrected pattern standard deviation (CPSD) at baseline treatment was 10.93± 2.12dB, while CPSD after GBE was 8.13±2.12dB (t=9.89, P=0.0001, Chi-square test). No significant changes were found in intraocular pressure, blood pressure, or heart rate after placebo or ginkgo biloba extract (GBE) treatment. Ginkgo biloba extract administration appeared to improve preexisting visual field damage in some patients with NTG[62].

There are many reasons for the disappointing clinical performance by neuroprotective compounds. The main one is that there are too many agents involved in the secondary degeneration of neuron, and reducing the harmful effects of one of them is insufficient to produce meaningful clinical effects. Further more, all of the agents that induce secondary degeneration are compounds intimately involved with the normal functioning of the nervous system. Therefore, in order to achieve clinically relevant neuroprotection, the activities of some of the agents of secondary degeneration would have to be blocked to the right extent and with the appropriate timing[45].

Immunologic Neuroprotection In glaucoma, some fibers which have already started to degenerate are enough to trigger a self-propagating chain of events, causing degeneration of adjacent fibers that were not directly damaged. Findings in Schwartz's lab suggested that in the case of damage resulting from an insult that does not involve pathogens, a protective mechanism operated by evoking an immune response against self, i.e. protective autoimmunity[63].

It is known that myelin basic protein (MBP) can cause experimental autoimmune encephalomyelitis. Injured optic nerves treated by passive transfer of T cells directed against MBP were found to possess two-to three-fold healthier fibers than control injured nerves, suggesting that the anti-MBP T cells have a protective effect on the damaged optic nerve. In an attempt to boost the immune neuropro-tection without inducing autoimmune disease, T cells directed against a cryptic epitope of MBP were used, and similar neuroprotective efficacy was observed[63].

And recently passive T cell transfer was tried to be replaced by active immunization. A synthetic copolymer, Cop-1, a FDA-approved drug for multiple sclerosis was chosen as the antigen. Vaccination of rats and mice with this compound after CNS insult induced protective immunity without the side effect of autoimmune disease. The benefit was manifested by a significantly increased survival rate of retinal ganglion cells in rat and mouse models of optic nerve crush injury, glutamate toxicity injected intraocularly, and chronically increased intraocular pressure[63].

The mechanism of T-cell-mediated neuroprotection is not yet known. One possible mechanism is that the T cells provide a source of neurotrophic factors, including nerve growth factor (NGF), neurotrophin (NT)-3, NT-4, NT-5, and brain-derived neurotrophic factor (BDNF). Using a benign protective autoimmunity may become a novel approach for the protection of neurons from secondary degeneration[63].

However, glaucoma may be associated with autoimmune processes. Elevated antiphosphatidylserin antibodies were found in normal tension glaucoma [64]. Complex IgG autoantibody repertoires against optic nerve antigens could be found in glaucomatous subjects and the NTG group had the highest variance from controls [65]. Tezel *et al* [66] hypothesized that one form of glaucoma may be an autoimmune neuropathy in which an individual's immune system is not only inappropriately regulated, but a cytotoxic effect is rendered by the very system which normally serves to protect it against stress.

So whether 'protective autoimmunity' can do benefit to glaucoma patients is not sure.

Stem Cell Therapy Stem cells are undifferentiated cells able to divide indefinitely yet maintain the ability to differentiate into specific cell types. They are able to survive throughout the lifetime of the organism, while maintaining their number, producing populations of daughter cells (transit amplifying cells) that can proceed down unique pathways of differentiation. Stem cells may be obtained from embryonic tissues, umbilical cord blood, and some differentiated adult tissues. Although the potential for stem cell based therapies for a variety of human diseases is promising, numerous problems remain to be overcome, such as methods for obtaining, transplanting, inducing differentiation, developing function, and eliminating immune reactions. Stem cells have a great potential value in treating eye diseases characterized by irreversible loss of cells, such as glaucoma[67].

There are at least 3 potential targets for stem cell therapy in glaucoma: the RGC, the ONH and the trabecular meshwork. So far, most work has focused on replacing RGCs because their death is the final common pathway for visual loss in glaucoma and other optic neuropathies. Because human

RGCs are mammalian CNS neurons that cannot divide and differentiate to replace other cells lost from disease, blindness from glaucoma is irreversible. Finding a way to differentiate stem cells into RGCs and allow them to connect to their appropriate targets would be a major step in repopulating the neurons lost in glaucoma. The main issues to be resolved are the survival and differentiation of the stem cells, maintaining the state of the surrounding microenvironment, extension of axons into the optic nerve, establishment of functional connections in the lateral geniculate nucleus, and appropriate activation of transsynaptically connected cortical targets. The RGC precursor cells introduced into the retina extend processes into the optic nerve head. The need to establish a functional network communicating information to the brain makes the problem of stem cell replacement of RGCs especially complex. However, because patients lose a substantial portion of their RGCs before developing functional deficits, there is hope that a limited amount of restoration might have a large effect on visual capability[67].

MISCELLANEOUS

Sex Recent literature suggests that sex may be important at least as it relates to the onset of glaucoma and choice of therapy[68]. The Rotterdam Study noted that women who were postmenopausal before 45 years of age were at a high risk of developing glaucoma compared with those who were older. In this study, the authors defined glaucoma as a characteristic glaucoma field defect and optic nerve finding. So the authors suggested a protective benefit of endogenous estrogen [69]. The effect of hormonal influences on ocular physiology has been stated in the literature. Observations during pregnancy include an improvement in outflow facility, ocular hypotension, and a decreased in aqueous production. Moreover, even among premenopausal women who are not pregnant, there may be changes related to their menstrual cycle [68]. During 6-month hormone therapy in postmenopausal women with normal-tension glaucoma, color Doppler parameters of ophthalmic arteries significantly improved. It seemed that hormone therapy beneficially affect the ocular vascularization [70]. Finally, in view of the higher prevalence of coronary artery disease among postmenopausal women, it is wise to choose a topical drug that will not exacerbate this problem[68].

Hypothyroidism In 1920, Hertel reported 2 patients in whom IOP control was improved with thyroid hormone replacement. It has been hypothesized that hypothyroidism is associated with the deposition of mucopolysaccharides in the trabecular meshwork with evidence from postmortem studies using human and animal eyes. Recently, in a large cohort of patients, a significantly greater risk is demonstrated of subjects with a preexisting diagnosis of hypothyroidism developing glaucoma, compared with controls[71].

Table 1 Ocular hypotension

Action mechanism	Possible development of novel drugs
Induction of MMPs	IL-1 α
	AP-1 stimulants:
	β -naphthofla
	3-methylcholanthrene
	tert-butylhydroquinone
Contraction of TM cells	H-7
Inhibition of AH secretion	A3R inhibitors
	MRS1191
	OT-7999
Activation of CB1	Cannabinoids

Table 2 Improvement of ocular blood flow

Action mechanism	Possible development of new drugs
Blockade of Ca-channel	Nimodipine
Inhibition of endothelin-1	Unoprostone
Acceleration of retinal circulation	Carbonic anhydrase inhibitors

CONCLUSION

Because of the advancement in research technologies, the etiology of glaucoma becomes much more complex than before. As a result, new drugs developed rapidly with novel ideas of action mechanisms. Although the end results are still within the current three classes of drug actions, the action mechanisms are entirely different. The major advantage of this development is to find better drugs with higher therapeutic indexes.

The most classic drugs under development are to lower the IOP in order to reduce the mechanical damage of ONH and to improve retinal blood flow for preserving the RGC (Table 1). To achieve these goals numerous agents are invented to lower the IOP through ① induction of MMPs [20-22], ② contraction of TM cells [23], ③ inhibition of AH secretion[24-27], and ④ activation of CB1 receptor [28,29]. These agents are at various stages of development both at preclinical and/or clinical phases. It is highly possible that some of these agents will be put in the clinic sometime in the future.

The second class of drugs under development are closely related to the first class as the reduction of IOP is intended to improve the OBF particularly in retina and ONH. However, some drugs do improve the ocular blood flow irrespective of the IOP changes (Table 2). These agents could be quite useful for the treatment of normo- tensive or low-tension glaucoma. Most agents are intended to relax the ocular vasculature to facilitate the blood flow[32,33,36-41].

Neuroprotection is the latest developed mechanisms of glaucoma treatment. Although the history of neuroprotection research is very short, there are a lot of agents under investigation in this class (Table 3). They include ① blockade of NMDA receptor [48-51], ② neurotrophic agents [45,56,57], ③ inhibition of iNOS [58], ④ inhibition of capases [59, 60], ⑤ protective autoimmunity [63], and ⑥ stem cell therapy [67], to name a few.

Table 3 Protection of ocular neurons

Action mechanism	Possible development of neuroprotectors
Blockade of NMDA receptor	MK-801
	Memantine
Replenishment of neurotransmitters	Apraclonidine
	Brimonidine
Blockade of Ca-channel	Flunarizine
	Lomerizine
	Nimodipine
Neurotrophic agents	BDNF
	Trk receptor agonists
	P75 receptor antagonists
Inhibition of iNOS	Aminoguanidine
Inhibition of caspases	T-588
Herbal medicine	Ginkgo biloba extract
Protective autoimmunity	Cop-1
Stem cell therapy	Stem cells

Even though there are many antiglaucoma drugs available in the clinic today, there is much room to be improved ① to reduce the side effects, ② to treat drug resistant glaucoma, ③ to improve the efficiency of the drugs, *etc*. Since all drugs for glaucoma treatment are used to stabilize the disease rather than to cure it, it is critical that an ideal drug with high therapeutic index and low cost price should be invented[4-8].

REFERENCES

1 The AGIS Investigators. The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol* 2000;130(4):429-440

2 Collaborative Normal-Tension Glaucoma Study Group. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. *Am J Ophthalmol* 1998;126(4):487-497

3 Kass MA, Heuer DK, Higginbotham EJ, for the Ocular Hypertension Treatment Study Group. The Ocular Hypertension Treatment Study: A randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol* 2002;120(6):701-713

4 Khaw PT, Shah P, Elkington AR. Glaucoma--2: treatment. *BMJ* 2004;328 (7432):156-158

5 Woodward DF, Gil DW. The inflow and outflow of anti-glaucoma drugs. *Trends Pharmacol Sci* 2004;25(5):238-241

6 Skuta GL. Should beta blockers be abandoned as initial monotherapy in chronic open angle glaucoma? View 2. *Br J Ophthalmol* 2002;86 (6):693-694; discussion 695

7 Anton A. Should beta blockers be abandoned as initial monotherapy in chronic open angle glaucoma? View 1. *Br J Ophthalmol* 2002;86 (6):692-693; discussion 695

8 Goldberg I. Should beta blockers be abandoned as initial monotherapy in chronic open angle glaucoma? The controversy. *Br J Ophthalmol* 2002;86(6):691-692

9 Brubaker RF. Targeting outflow facility in glaucoma management. *Surv Ophthalmol* 2003;48(Suppl 1):17-20

10 Kiland JA, Gabelt B, Tand Kaufman PL. Studies on the mechanism of action of timolol and on the effects of suppression and redirection of aqueous flow on outflow facility. *Exp Eye Res* 2004;78(3):639-651

11 Krauss AH, Woodward DF. Update on the mechanism of action of bimatoprost: a review and discussion of new evidence. *Surv Ophthalmol* 2004;49(Suppl 1):5-11

12 Brubaker RF, Schoff EO, Nau CB, Carpenter SP, Chen K, Vandenburgh AM. Effects of AGN 192024, a new ocular hypotensive agent, on aqueous dynamics. *Am J Ophthalmol* 2001;131(1):19-24

13 Richter M, Krauss AH, Woodward DF, Lütjen-Drecoll E. Morphological changes in the anterior eye segment after long-term treatment with different receptor selective prostaglandin agonists and a prostamide. *Invest Ophthalmol Vis Sci* 2003;44(10):4419-4426

14 Katz LJ. Bimatoprost: establishing its role in glaucoma management. *Surv Ophthalmol* 2004;49(Suppl 1):1-4

15 Lütjen-Drecoll E. Functional morphology of the trabecular meshwork in primate eyes. *Prog Retin Eye Res* 1999;18(1):91-119

16 Yue BY. The extracellular matrix and its modulation in the trabecular meshwork. *Surv Ophthalmol* 1996;40(5):379-390

17 Cavallotti C, Feher J, Pescosolido N, Sagnelli P. Glycosaminoglycans in human trabecular meshwork: age-related changes. *Ophthalmic Res* 2004;36(4):211-217

18 Li A, Tane N, Roy S. Fibronectin overexpression inhibits trabecular meshwork cell monolayer permeability. *Mol Vis* 2004;10:750-757

19 La Rosa, Francis A, Lee DA. Collagen degradation in glaucoma: will it gain a therapeutic value? *Curr Opin Ophthalmol* 2000;11(2):90-93

20 Pang IH, Hellberg PE, Fleenor DL, Jacobson N, Clark AF. Expression of matrix metalloproteinases and their inhibitors in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 2003;44(8):3485-3493

21 Pang IH, Fleenor DL, Hellberg PE, Stropki K, McCartney MD, Clark AF. Aqueous outflow-enhancing effect of tert-butylhydroquinone: involvement of AP-1 activation and MMP-3 expression. *Invest Ophthalmol Vis Sci* 2003;44(8): 3502-3510

22 Fleenor DL, Pang IH, Clark AF. Involvement of AP-1 in interleukin-1alpha-stimulated MMP-3 expression in human trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 2003;44(8):3494-3501

23 Tian B, Wang RF, Podos SM, Kaufman PL l. Effects of topical H-7 on outflow facility, intraocular pressure, and corneal thickness in monkeys. *Arch Ophthalmol* 2004;122(8):1171-1177

24 Civan MM, Macknight AD. The ins and outs of aqueous humour secretion. *Exp Eye Res* 2004;78(3):625-631

25 Mitchell CH, Peterson-Yantorno K, Carre DA, McGlinn AM, Coca-Prados M, Stone RA, Civan MM. A3 adenosine receptors regulate Cl^- channels of nonpigmented ciliary epithelial cells. *Am J Physiol* 1999;276(3 Pt 1):659-666

26 Avila MY, Stone RA, Civan MM. Knockout of A3 adenosine receptors reduces mouse intraocular pressure. *Invest Ophthalmol Vis Sci* 2002;43(9):3021-3026

27 Okamura T, Kurogi Y, Hashimoto K. Structure-activity relationships of adenosine A3 receptor ligands: new potential therapy for the treatment of glaucoma. *Bioorg Med Chem Lett* 2004;14(14):3775-3779

28 Shi C, Szczesniak A, Mao L, Jollimore C, Coca-Prados M, Hung O, Kelly ME. A3 adenosine and CB1 receptors activate a PKC-sensitive Cl^- current in human nonpigmented ciliary epithelial cells via a G beta gamma-coupled MAPK signaling pathway. *Br J Pharmacol* 2003;139(3):475-486

29 Tomide I, Pertwee RG, Azuara-Blanco A. Cannabinoids and glaucoma. *Br J Ophthalmol* 2004;88(5):708-713

30 Heijl A, Leske MC, Bengtsson B, Bengtsson B, Hussein M, Early Manifest Glaucoma Trial Group. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Arch Ophthalmol* 2002;120(10):1268-1279

31 Leske MC, Heijl A, Hussein M, Bengtsson B, Hyman L, Komaroff E, Early Manifest Glaucoma Trial Group. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. *Arch Ophthalmol* 2003;121(1):48-56

32 Flammer J, Orgul S, Costa VP, Orzalesi N, Krieglstein GK, Serra LM, Renard JP, Stefansson E. The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res* 2002;21(4):359-393

33 Costa VP, Harris A, Stefansson E, Flammer J, Krieglstein GK, Orzalesi N, Heijl A, Renard JP, Serra LM. The effects of antiglaucoma and systemic medications on ocular blood flow. *Prog Retin Eye Res* 2003;22(6):769-805

34 Oku H, Sugiyama T, Kojima S, Watanabe T, Azuma I. Experimental optic cup enlargement caused by endothelin-1-induced chronic optic nerve head ischemia. *Surv Ophthalmol* 1999;44(Suppl-1):74-84

35 Chauhan BC, LeVatte TL, Jollimore CA, Yu PK, Reitsamer HA, Kelly ME, Yu DY, Tremblay F, Archibald ML. Model of endothelin-1-induced chronic optic

neuropathy in rat. *Invest Ophthalmol Vis Sci* 2004;45(1):144-152
36 Sugiyama T, Azuma I. Effect of UF-021 on optic nerve head circulation in rabbits. *Jpn J Ophthalmol* 1995;39(2):124-129
37 Polska E, Doelemeyer A, Luksch A, Ehrlich P, Kaehler N, Percicot CL, Lambrou GN, Schmetterer L. Partial antagonism of endothelin 1-induced vasoconstriction in the human choroid by topical unoprostone isopropyl. *Arch Ophthalmol* 2002;120(3):348-352
38 Kitaya N, Yoshida A, Ishiko S, Mori F, Abiko T, Ogasawara H, Kato Y, Nagaoka T. Effect of timolol and UF-021 (a prostaglandin-related compound) on pulsatile ocular blood flow in normal volunteers. *Ophthalmic Res* 1997;29(3):139-144
39 Beano F, Orgul S, Stumpfig D, Gugleta K, Flammer J. An evaluation of the effect of unoprostone isopropyl 0.15% on ocular hemodynamics in normal-tension glaucoma patients. *Graefes Arch Clin Exp Ophthalmol* 2001;239(2):81-86
40 Tamaki Y, Araie M, Tomita K, Nagahara M, Sandoh S, Tomidokoro A. Effect of topical unoprostone on circulation of human optic nerve head and retina. *J Ocul Pharmacol Ther* 2001;17(6):517-527
41 Makimoto Y, Sugiyama T, Kojima S, Azuma I. Long-term effect of topically applied isopropyl unoprostone on microcirculation in the human ocular fundus. *Jpn J Ophthalmol* 2002;46(1):31-35
42 Luksch A, Rainer G, Koyuncu D, Ehrlich P, Maca T, Gschwandtner ME, Vass C, Schmetterer L. Effect of nimodipine on ocular blood flow and colour contrast sensitivity in patients with normal tension glaucoma. *Br J Ophthalmol* 2005;89(1):21-25
43 Michalk F, Michelson G, Harazny J, Werner U, Daniel WG, Werner D. Single-dose nimodipine normalizes impaired retinal circulation in normal tension glaucoma. *J Glaucoma* 2004;13(2):158-162
44 Boehm AG, Breidenbach KA, Pillunat LE, Bernd AS, Mueller MF, Koeller AU. Visual function and perfusion of the optic nerve head after application of centrally acting calcium-channel blockers. *Graefes Arch Clin Exp Ophthalmol* 2003;241(1):34-38
45 Barkana Y, Belkin M. Neuroprotection in ophthalmology: a review. *Brain Res Bull* 2004;62(6):447-453
46 Goldblum D, Mittag T. Prospects for relevant glaucoma models with retinal ganglion cell damage in the rodent eye. *Vision Res* 2002;42(4):471-478
47 Kerrigan LA, Zack DJ, Quigley HA, Smith SD, Pease ME. TUNEL-positive ganglion cells in human primary open-angle glaucoma. *Arch Ophthalmol* 1997;115(8):1031-1035
48 Yoles E, Muller S, Schwartz M. NMDA-receptor antagonist protects neurons from secondary degeneration after partial optic nerve crush. *J Neurotrauma* 1997;14(9):665-675
49 Lipton SA. Possible role for memantine in protecting retinal ganglion cells from glaucomatous damage. *Surv Ophthalmol* 2003;48(Suppl-1):38-46
50 Hare WA, WoldeMussie E, Lai RK, Ton H, Ruiz G, Chun T, Wheeler L. Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, I: Functional measures. *Invest Ophthalmol Vis Sci* 2004;45(8):2625-2639
51 Hare WA, WoldeMussie E, Weinreb RN, Ton H, Ruiz G, Wijono M, Feldmann B, Zangwill L, Wheeler L. Efficacy and safety of memantine treatment for reduction of changes associated with experimental glaucoma in monkey, II: Structural measures. *Invest Ophthalmol Vis Sci* 2004;45(8):2640-2651
52 Wheeler L, WoldeMussie E, Lai R. Role of alpha-2 agonists in neuroprotection. *Surv Ophthalmol* 2003;48(Suppl-1):47-51
53 Boehm AG, Breidenbach KA, Pillunat LE, Bernd AS, Mueller MF, Koeller AU. Visual function and perfusion of the optic nerve head after application of centrally acting calcium-channel blockers. *Graefes Arch Clin Exp Ophthalmol* 2003;241(1):34-38
54 Michalk F, Michelson G, Harazny J, Werner U, Daniel WG, Werner D. Single-dose nimodipine normalizes impaired retinal circulation in normal tension glaucoma. *J Glaucoma* 2004;13(2):158-162
55 Netland PA, Chaturvedi N, Dreyer EB. Calcium channel blockers in the management of low-tension and open-angle glaucoma. *Am J Ophthalmol* 1993;115(5):608-613
56 Martin KR, Quigley HA, Zack DJ, Levkovitch-Verbin H, Kielczewski J, Valenta D, Baumrind LA, Pease ME, Klein RL, Hauswirth WW. Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model. *Invest Ophthalmol Vis Sci* 2003;44(10):4357-4365
57 Rudzinski M, Wong TP, Saragovi HU. Changes in retinal expression of neurotrophins and neurotrophin receptors induced by ocular hypertension. *J Neurobiol* 2004;58(3):341-354
58 Neufeld AH. Pharmacologic neuroprotection with an inhibitor of nitric oxide synthase for the treatment of glaucoma. *Brain Res Bull* 2004;62(6):455-459
59 McKinnon SJ. Glaucoma: ocular Alzheimer's disease? *Front Biosci* 2003;8:1140-1156
60 Maeda K, Sawada A, Matsubara M, Nakai Y, Hara A, Yamamoto T. A novel neuroprotectant against retinal ganglion cell damage in a glaucoma model and an optic nerve crush model in the rat. *Invest Ophthalmol Vis Sci* 2004;45(3):851-856
61 Hirooka K, Tokuda M, Miyamoto O, Itano T, Baba T, Shiraga F. The Ginkgo biloba extract (Egb 761) provides a neuroprotective effect on retinal ganglion cells in a rat model of chronic glaucoma. *Curr Eye Res* 2004;28(3):153-157
62 Quaranta L, Bettelli S, Uva MG, Semeraro F, Turano R, Gandolfo E. Effect of Ginkgo biloba extract on preexisting visual field damage in normal tension glaucoma. *Ophthalmology* 2003;110(2):359-362; discussion 362-364
63 Schwartz M. Vaccination for glaucoma: dream or reality? *Brain Res Bull* 2004;62(6):481-484
64 Kremmer S, Kreuzfelder E, Klein R. Bontke N, Henneberg-Quester KB, Steuhl KP, Grosse-Wilde H. Antiphosphatidylserine antibodies are elevated in normal tension glaucoma. *Clin Exp Immunol* 2001;125(2):211-215
65 Grus FH, Joachim SC, Hoffmann EM, Pfeiffer N. Complex autoantibody repertoires in patients with glaucoma. *Mol Vis* 2004;10:132-137
66 Tezel G, Yang J, Wax MB. Heat shock proteins, immunity and glaucoma. *Brain Res Bull* 2004;62(6):473-480
67 Levin LA, Ritch R, Richad JE, Borras T. Stem cell therapy for ocular disorders. *Arch Ophthalmol* 2004;122(4):621-627
68 Higginbotham EJ. Does sex matter in glaucoma? *Arch Ophthalmol* 2004;122(3):374-375
69 Hulsman CA, Westendorp IC, Ramrattan RS, Wolfs RC, Witteman JC, Vingerling JR, Hofman A, de Jong PT. Is open-angle glaucoma associated with early menopause? The Rotterdam Study. *Am J Epidemiol* 2001;154(2):138-144
70 Battaglia C, Mancini F, Regnani G, Persico N, Volpe A, De Aloysio D. Hormone therapy and ophthalmic artery blood flow changes in women with primary open-angle glaucoma. *Menopause* 2004;11(1):69-77
71 Girkin CA, McGwin G Jr, McNeal SF, Lee PP, Owsley C. Hypothyroidism and the development of open-angle glaucoma in a male population. *Ophthalmology* 2004;111(9):1649-1652