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Ginkgo Biloba Extract in Ophthalmic and Systemic Disease, With a Focus on Normal-Tension Glaucoma

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Abstract: Glaucoma is a neurodegenerative eye disease that results in retinal ganglion cell loss and ultimately loss of vision. Elevated intraocular pressure (IOP) is the most common known risk factor for retinal ganglion cell damage and visual field loss, and the only modifiable risk factor proven to reduce the development and progression of glaucoma. This has greatly influenced our approach and assessment in terms of diagnosis and treatment. However, as many as ≥50% of patients with progressive vision loss from primary open angle glaucoma without IOP elevation (≤22 mm Hg) have been reported in the United States and Canada; 90% in Japan and 80% in Korea. Extensive research is currently underway to identify the etiology of risk factors for glaucoma other than or in addition to elevated IOP (so-called "normal-tension" glaucoma; NTG) and use this knowledge to expand available treatment options. Currently, Food and Drug Administration-approved medications for glaucoma exclusively target elevated IOP, suggesting the need for additional approaches to treatment options beyond the current scope as the definition of glaucoma changes to encompass cellular and molecular mechanisms. This review focuses on alternative medical approaches, specifically Ginkgo Biloba extract, as a potential treatment option for normal-tension glaucoma.

Key Words: ginkgo, glaucoma, normal-tension, supplements

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INTRODUCTION

Glaucoma is a highly prevalent neurodegenerative condition of the optic nerve (and thus is actually a brain disease) and is the leading cause of irreversible blindness worldwide. Vascular insufficiency, expressed as reduced mean ocular perfusion pressure, is a prominent feature of normal-tension glaucoma (NTG) and leads to progressive death of retinal ganglion cells (RGCs), resulting in progressive thinning of the nerve fiber layer and irreversible vision loss.[1,2] Despite the diverse etiology of glaucoma, the only proven approach to treatment centers around lowering IOP with medical, laser, and surgical interventions, which decreases vascular resistance, increasing mean vascular flow. These treatments, however, are not effective in cases of nonelevated IOP etiology.

In addition to elevated IOP, other risk factors, including systemic diseases, low intracranial pressure, genetic factors, and factors associated with neurodegenerative diseases such as mitochondrial dysfunction, oxidative damage, excitotoxicity, and microglial activation can predispose individuals to glaucomatous optic nerve damage. The only Food and Drug Administration (FDA)-approved treatment addressing the vascular risk factors are calcium channel blockers, but their efficacy has been questioned.[3] Given this, new pharmaceutical approaches that alter causative genetic, biochemical, cell biological, and pathophysiologic mechanisms, many of which remain to be discovered, and agents, which provide neuroprotection, must be explored to target non-IOP-dependent risk factors.

Nonpharmaceutical medicines (herbal extracts, alternative medicine) have been used by every society since before recorded history and are also used by animals other than humans. Formerly derided after the growth of pharmaceutical medicine, these are now being widely sought after by pharmaceutical companies worldwide. The earliest of these recorded in the first textbook of Chinese medicine approximately 5000 years ago is *Ginkgo biloba* extract (GBE).[4,5] In this review, we will discuss physiological and molecular mechanisms of GBE and its effectiveness in light of our enhanced understanding of glaucoma.

NTG

The etiology of NTG is associated with systemic conditions such as ischemic migraine, atrial fibrillation, intermittent claudication, Raynaud, Flammer syndrome, and obstructive sleep apnea syndrome (OSAS).[4,6–9] These conditions have been characterized by remodeling and degeneration of the ONH,[10] and metabolic dysregulation that is especially damaging in locations of the body with deep layer microvasculature, such as in the eye.[11–13]

Increased risk for NTG has been identified in patients with low blood pressure, autonomic dysfunction, and cardiovascular disease.[14–16] Some patients with NTG have abnormal hemorheological parameters that result in lower oxygen transport efficiency, leading to decreased microperfusion and decreased perfusion to the ONH.[17]

PLANT-BASED MEDICINE

The use of medicinal plants precedes human societies. Chimpanzees in the wild eat bitter-tasting *Vernonia* species that contain steroid-related compounds, which are known for their

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antiparasitic activity and stimulation of uterine contractions and eaten by pregnant females.[18,19] Orangutans self-medicate themselves with a paste derived from *Dracaena cantleyi*, which inhibits tumor necrosis factor (TNF)-α induced inflammatory cytokines, by rubbing it in locations of pain.[20] As chronic medical conditions have many common underlying pathological features that can be targeted, a large number of nonpharmaceutical agents have been studied for their neuroprotective, anti-inflammatory, anti-oxidative, and other properties.[21]

GBE

Historic Significance

Ginkgo biloba belongs to the first order of true trees, originating during the Permian Era about 250 million years ago. In Asian cultures, specifically those of China and Japan, these trees are widely cultivated and the seeds are used for both food and medicinal purposes. GBE was originally used in Chinese traditional medicine as treatment in a multitude of medical conditions and symptoms.[4]

GBE was first introduced to the European market by Dr. Willmar Schwabe's preparation in 1965, known as EGb 761, which is a current criterion standard.[22,23] Research trials followed thereafter to evaluate the therapeutic effects of GBE through both basic and clinical science research. EGb 761 was modified to maximize the percentage of active ingredients, by increasing percent fraction of flavonoid and terpene compounds and minimizing ginkgolic acid to concentrations $<0.0005\%$ to avoid the allergenic and genotoxic effects associated with this substance.[24,25]

Ingredients

GBE is a leaf extract and its composition is not held responsible to FDA's standards; therefore, its chemical composition can vary. Commercially available GBE consists of 60 bioactive compounds and is a sole source of about 30 of them.[26,27] In the most widely used EGb 761 extract, the 2 major component groups are flavonoids and terpenoids. Flavonoids constitute 24% to 27% of the extract, examples of which are biflavones, catechin derivatives, flavonol glycosides, and 7% proanthocyanidins. Terpenoids make up 5% to 7% of the extract with examples of bilobalide; ginkgolide A; ginkgolide B, ginkgolide C; ginkgolide J. The extract also contains alkylphenols (ginkgolic acids) and organic acids.[26,27]

Vasoactive Effects

Nitric oxide (NO) has several functions depending on the cellular environment surrounding its release. Under normal physiologic conditions, GBE increases NO levels, leading to vasodilation and an increase in blood flow. Additionally, it upregulates gene expression, activating enzymes for NO synthesis (eNOS) and modulating molecular pathways that culminate in NO production.[28,29] NO regulates blood flow through vasodilatory molecules such as bradykinin, histamine, acetylcholine, substance P, and insulin. Upon activation, eNOS has an increased affinity for calcium, which results in increased muscle contractility, and further vasodilation.[28,29]

During an oxygen-deprived, ischemic state, where NO mediates neurotoxicity through neurodegeneration and apoptosis, EGb 761 inhibits the synthesis of NO through inducible NO synthase (iNOS) enzyme inhibition and downregulation of its nuclear factor (NF)-κB transcription factor.[30–32]

GBE also decreases endothelin-1, another molecule with vasoconstrictive properties.[33] Systemically, GBE's regulation of vasoactive substances increased both systolic and diastolic peak velocity measures.[33] Flavones inhibit phosphodiesterase-5, which is a property leveraged in antihypertensive drugs, as it has a vasorelaxant effect.[28,34,35] Another major impact on vasculature occurs through the renin–angiotensin pathway, which induces vasoconstriction. GBE decreases renin release by inhibiting prostaglandin PGI2, which positively induces renin.[35] Meanwhile, clinical studies, using dynamic susceptibility contrast magneic resonance imaging, showed that GBE increased global cerebral blood flow.[36]

Hemorrheological Regulation

Abnormal hemorheological properties contribute to the development of microvascular diseases such as microangiopathies seen in diabetes mellitus (DM). GBE impacts the hemorheological properties of blood by promoting erythrocyte deformability, and improving blood viscosity.[37] GBE has a strong fibrinolytic effect equivalent to that of streptokinase, decreasing fibrinogen levels important for clotting.[37,38] These changes improve blood perfusion, as shown by increased blood flow rates in the retinal capillaries of patients with diabetic retinopathy by altering hemorheological blood parameters.[37]

Anti-inflammatory Effects

On a molecular level, the inflammatory response is attributed to TNF-α activating an inflammatory cascade by enhancing the activity of leukocytes, neutrophils, monocytes, and increasing endothelial adhesion. GBE suppresses TNF-α by regulating the protein-1 signaling pathway.[39]

Prostaglandin E2 (PGE2) contributes significantly to inflammatory-based diseases.[40] GBE suppresses PGE2 levels by downregulating cyclooxygenase-2 (COX-2) expression, which is responsible for producing prostaglandins from arachidonic acid. One transcription factor that regulates COX-2 expression is NF-kB. NF-kB is a transcription factor that regulates many genes that are involved in the inflammatory process, including COX-2, NO synthase, and TNF-α.[40] By downregulating NF-kB, GBE is thus able to downregulate PGE2.[31,41]

Cytokines are the main mediators of signaling during the inflammatory cascade. GBE decreased MIP-2 and MCP-1 cytokine levels, resulting in an anti-inflammatory effect.[40] Zhang et al in 2018 showed that GBE suppresses cytokine signaling 2, which further suppressed the inflammatory response.[42]

Anti-oxidative Effects

Flavonoids have free radical scavenging activity targeting reactive oxygen species (ROS), hydroxyl, superoxide peroxyl, hydroxyl radicals, and reactive nitrogen species such as NO and ferryl ion species.[43,44] Terpenoids, however, inhibit free radical release. Bilobalide and ginkgolide constituents of terpenoids increase reducing enzymes such as superoxide dismutase, glutathione peroxidase, catalase, heme-oxygenase-1 activity that process free radical molecules. Decreasing circulating free radicals reduce lipid peroxidation, erythrocyte malonaldehyde levels, decrease endothelial adhesive properties, reducing membrane peroxidation, while preserving its fluidity and integrity.[45]

Neuroprotective

Ischemia-reperfusion Injury

GBE is a multifunctional neuroprotective agent, and the protection is associated with activation of the Heme oxygenase 1 (HO1)/Nrf2 pathway, which has antioxidant effects; it promotes neurite growth and angiogenesis by upregulating vascular endothelial growth factor (VEGF).[24,46–48] In addition, animals used that received EGb 761 in this study before ischemic induction had an increased number of activated astrocytes and microglia, further indicating its neuroprotective effect.[24,46,49–51]

Other studies determined ginkgolide B (GKB) to be neuroprotective post-ischemic induction by increasing nestin, an intermediate filamentous protein lining the ventricles, and inducing neuron specific-enolase, which partakes in glycolysis.[52] Wu et al demonstrated that GKB inhibits expression of stress-related protein RTP801, thereby decreasing post-ischemia-reperfusion injury stress.[53]

One neuroprotective hypothesis that has been suggested is the idea of preconditioning post-hypoxic-ischemic injury. In vitro studies showed that GBE pre-treatment before ischemic onset had the same effect as hypoxic preconditioning, both resulting in upregulation of p-glycogen synthase kinase (p-GSK), p-extracellular receptor kinase (p-ERK)/t-ERK, hypoxia-inducible factor-1α, and erythropoietin expression.[23,54]

Neuronal Differentiation and Protection

GBE provides neuroprotection against ROS, calcium overload, negative effects of NO signaling in apoptosis, and beta-amyloid-induced toxicity.[55] GKB administration was associated with an increase in neuronal and astrocytic markers such as brain-derived epidermal factor and epidermal growth factor.[52] Cai et al cotransplanted neural stem cells with astrocytes and brain microvascular endothelial cells in rodents, leading to memory improvement post-stroke.[51]

Suppression of cytokine signaling (SOCS2) pertaining to neurite growth was positively impacted by GKB administration and has been attributed to JAK/STAT signaling pathway.[56,57] SOCS2 also has been shown to bind Epidermal Growth Factor receptor, which stimulated neuronal differentiation and neurite growth. Behaviorally, these molecular changes were paralleled by improvements in neurological function.[42]

RGC death has been the underlying cause of neurodegenerative diseases tied to hypoxia, glutamate toxicity, and oxidative stress. Flavonoids such as nicotiflorin, rutin, and quercetin increased RGC survival rate. Immunoreactivity assays showed that rutin inhibited caspase-3 under hypoxia and glutamate stress conditions, thereby decreasing cell death.[55,58] It has been suggested that there is a time window between the decrease in function before RGC death when GBE intervention is effective.[55]

GBE produces a 3-fold increase in expression of the transthyretin gene, which sequesters amyloid-β (Aβ) protein in vitro, resisting Aβ aggregation, and prevents amyloid formation directly and through adaptor proteins that interact with Alzheimer β-amyloid precursor protein.[59] Patients taking GBE improved in cognitive abilities, global functional, and behavioral outcomes.[60,61] GBE was most beneficial for patients with neuropsychiatric comorbidities in addition to cognitive decline, which are frequently seen in dementia.[62] Immunohistochemistry revealed that EGb 761 positively influenced molecular mechanisms underlying memory formation by modulating the expression of GAP-43, CREB-1, and GFAP.[63]

Metal Homeostasis

GBE maintains homeostasis of manganese (Mn) and copper (Cu) metals in the brain. Mn and Cu are prominent cofactors of antioxidant enzymes.[43,64] Homeostasis is important for proper function, and GBE regulates metal concentration to stay within a physiologically optimal range.[43,64]

Neurotransmitter Regulation

The bilobalide and ginkgolide components of GBE antagonize gamma-amino butyric acid and glycine inhibitory receptors, inducing an overall excitatory effect. Global excitation ultimately strengthens synapse formation. GBE upregulates excitatory receptors, such as voltage-gated calcium CACNG2 and chloride channels ClCN3 that are expressed in brain regions responsible for sensory, motor, and cognitive functions.[59]

Specifically, GBE upregulates gene expression of AMPA-2 or GluRB receptors, which is an ionotropic glutamate receptor responsible for synaptogenesis, memory formation, and learning.[59] GBE also demonstrated antagonistic activity on *N*-methyl-D-aspartate receptors from the same receptor class as AMPA-2. *N*-methyl-D-aspartate allows calcium ions (Ca^{2+}) inside the cells, thereby inducing a toxic environment for the cell. Glutamate neurotransmitter, although essential in interneuronal communication of inner retinal cells, is also toxic to neuronal ganglion cells in high concentrations by increasing intracellular calcium levels.[59]

Hormonal Alteration

GBE increases the expression of several hormones, such as thyroid hormone, growth hormone, and prolactin, which are essential for neuronal proliferation and differentiation, cognitive capacity related to memory, mental alertness, motivation, and working capacity. Thyroid hormone levels are increased through transthyretin, responsible in the transport of thyroid precursor, thyroxine.[59] Growth hormone deficiency is a hallmark of declined growth and decreased cognitive performance. GBE increased the expression of growth hormone in the cortex region. GBE reduced serum prolactin levels in rat models, suggesting that prolactin secretion is regulated through the dopaminergic system.[65] Increases in dopamine in the medial preoptic area and the arcuate nucleus have been related to enhancements of copulatory behavior accompanied by decreases in prolactin.[65]

Antineoplastic

Molecular Tumor Suppression and DNA Repair

GBE impacts expression of proteins involved in DNA damage signaling, repair, and gene expression through histone remodeling.[66,67] Certain flavonoids and terpenoids within GBE have antimutagenic properties, reducing substances such as ofloxacin and acridine orange by 99%.[68] EGb 761 can regulate the cell-cycle via ERK1/2 signaling that is implicated in gastric cancer.[69] GBE also interacts with steroidogenesis pathways and has aromatase activity that is prominent in breast cancer cells and sensitizing cells to antineoplastic drugs.[70–72] Additional cancer pathways impacted by GBE include apoptosis induction via p53 transcription factor, Akt, and NF-kB in melanoma.[73,74]

Longevity

Aging has been associated with many biomarkers, one of which is cell cycle halting and reduction in differentiation. GBE

produced neurogenic effects in elderly mice via a decrease in apoptotic cells that had activated caspase-3 markers, increased neural stem cells and production of new neurons.[75] GBE was also shown to regulate cell cycle progression through MAPK14 and CDK7 kinase enzymes.[76]

A key molecule associated with aging mechanisms is mammalian target of rapamycin. The inhibition of this pathway was noted to slow aging process in model organisms, and GBE was found to downregulate it.[77]

Proteins Regulation

Heat shock proteins are important for preserving the structural integrity of proteins by acting as chaperones that preserve protein conformation in stressful environments such as cold, heat, and ultraviolet (UV) exposure. It has been found that GBE downregulated a shock protein associated with metastasis in non-small lung cancer cell lines.[78]

Aglycon components of GBE exhibit proteasome inhibitory functions. Proteasomes are responsible for the degradation of proteins that are tagged with a ubiquitin molecular tag. In vitro studies in HL-60 cells, which are leukemia preprogrammed cells, revealed that aglycons inhibited chymotrypsin-like enzyme activity, contributing to the anticarcinogenic, antioxidative, anti-inflammatory, and neuroprotective activities.[79]

Mitochondrial-level Regulation

Electron Transport Chain

Flavonoids, with their hydrophobic acid chemical characteristics, increase proton permeability across the inner mitochondrial membrane, which results in uncoupling, decreasing free radical species.[80] Studies with EGb 761 showed beneficial effects on mitochondrial complexes I, IV, and V, and protected against nitrosative stress.[81] Given its vital function and prominent presence across the mitochondrial membrane, it is essential to maintain the viability of Na/K ATPase. The membrane density of Na/K ATPase is reduced in oxidative stress, and GBE has been shown to counteract this decrease.[82,83]

Antiapoptotic

Studies have demonstrated anti-apoptotic effects of GBE by preventing cathepsin-mediated cell death, inhibiting stress-activated protein kinase/c-Jun *N*-terminal kinase activation.[84,85] GBE led to blocking the mitochondrial apoptotic pathway, which is reflected in the directed downregulation of proapoptotic genes such as *Fas*, *Bax*, *Bcl-xS*, and *AT2* receptor genes.[86]

GBE AND SYSTEMIC EFFECTS

Systemic Diseases

GBE ameliorates conditions related to inflammation and immune hypersensitivity seen in asthma, ulcerative colitis, and inflammatory bowel disease. Patients with asthma given GBE had a significant decrease in interleukin-5, protein kinase C-α-positive inflammatory cells, eosinophils, and increased forced expiratory volume in 1 second.[87] GBE attenuated colon damage in ulcerative colitis and inflammatory bowel disease by decreasing myeloperoxidase activity, TNF-α, and interleukin-1β levels and increased glutathione concentration, which ameliorates oxidative and inflammatory responses that contribute to tissue fibrosis.[88]

GBE improved auditory function in cases of Meniere disease, exceeding anti-vertiginous drugs such as betahistine in its clinical effectiveness for patients with vertigo and Meniere disease.[89,90]

GBE was effective in controlling vitiligo spread by cessation of progression of pigment loss.[91,92] Patients with DM taking GBE had a decrease in HbA1c, fasting serum glucose, insulin, insulin resistance, visceral adiposity index, lipid profile, and inflammatory markers.[93,94] GBE contains endophytes, which after a 3-fold dilution of a CDW7 bioactive strain inhibited the mycelial growth and conidia germination of *Fusarium graminearum* pathogen.[95]

Effects on Vasculature

GBE has a well-documented influence on blood flow due to its vasodilatory properties. Given these identified effects, GBE exhibited protective effects against edema,[96] which is one of the major causes of cerebral ischemia. Cerebral ischemia can lead to headaches such as ischemic migraines, and clinical studies with GKB reduced migraines with typical aura or migraine aura without headache.[97]

GBE's vasoactive properties have been studied in coronary artery disease and peripheral vascular disease (PVD) diseases such as intermittent claudication, Raynaud phenomenon, obstructive OSAS, and erectile dysfunction. GBE improved outcomes in myocardial functional recovery, reduced the number of ventricular extrasystoles, reperfusion-induced ventricular tachycardia, and slowed myocardial stunning.[54,98,99] In cases of PVD, GBE increased claudication distance, measured as distance subjects walked without pain.[100] Muir et al[6] showed that GBE was effective in decreasing the number of Raynaud attacks that occur due to episodic vasospasms. It had a positive impact on OSAS by reducing corticotrophin-releasing hormone activity and thus the sympathetic activity, thereby reversibly increasing non-rapid eye movement sleep density.[7,13,101] GBE has also been tested in patients with erectile dysfunction, which is a frequent side-effect of antidepressant medication, showing significant improvements upon long-term administration.[102,103]

GBE attenuated diabetic atherosclerosis by counteracting endoplasmic reticulum stress with autophagy, which is significant in atherosclerosis. GBE ultimately resulted in lower cholesterol deposits by downregulating lectin-like oxidized low-density lipoproteins-receptor-1, NADPH oxidase 4, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1.[40,104,105] GBE flavonoids were beneficial in counteracting venous insufficiency in patients with varicosities,[106] which occur from hypoxia, inflammation, ROS generation, and oxidative stress.

GBE AND THE EYE

Retinal Diseases

The most prevalent diseases of the eye are neurodegenerative in nature. They include age-related macular degeneration (MD), retinal diseases, and glaucoma, and occur in the context of ischemia and oxidation. GBE offers potential in addressing these pathologies, given its properties of inducing lysosomal autophagy, thereby promoting clearance of neurodegenerative aggregates. [32,107] RGCs coalesce to form the ONH, projecting their axons to the brain, so that the degeneration of RGCs leads to optic nerve head abnormalities. The length of the axons makes them more susceptible to oxidative damage, radical damage, mechanical compression, and photo-oxidative damage.

The retina is highly susceptible to oxidative and free radical damage due to light exposure of photoreceptors, which are the main source of ROS production. Animal studies showed less apoptotic cells in the photoreceptor and outer nuclear layer, and increased survival of RGCs with GBE administration post either light-induced damage or ONH crushing.[108,109] Studies also suggested the potential use of GBE in retinitis pigmentosa that occurs from oxidative dysregulation.[110] EGb 761 treatment also decreased the frequency of retinal detachment, prevented inflammation related to retinal disease, and reduced inflammation in cases of uveitis.[40,111,112]

DM can manifest as microvascular damage to the eye and result in diabetic retinopathy, which is diagnosed in one-third of the population with DM.[113] In diabetic animal models, GBE decreased pathological molecular mechanisms that manifest as the breakdown of blood–retina barrier, exudates, hemorrhages, ischemia, and neovascularization.[31,114–119] The retina is very metabolically active and is, therefore, very sensitive to the ischemic conditions such as retinal vascular occlusion or diabetic retinopathy, vascular dysregulation, and atherosclerotic changes.[105,120] By increasing blood flow to the retina, EGb 761 can prevent retinal degenerative processes.[121]

MD and Cataracts

MD occurs due to mechanisms related to ischemia, oxidation, and free radical formation that lead to death of retinal pigment epithelium in the macula. GBE's neuroprotective functions showed improvement in dry and senile MD with its positive effects on retinal pigment epithelium and thus visual acuity.[122–127]

Oxidative stress plays an important role in cataractogenesis or lens opacification. Animal studies showed that 75% of rats on GBE did not develop cataracts and 25% showed minimal opacification compared with the control group.[128] This finding was attributed to increased levels of lenticular antioxidant enzyme activity, glutathione in its reduced form, and higher levels of malonaldehyde, indicative of an overall reduced molecular state.[128] Studies with streptozotocin-induced cataractogenesis in rat models showed that GBE, rutin, and quercetin delayed the progression of lens opacification with quercetin being most effective.[31,128]

GBE AND GLAUCOMA

RGC apoptosis in glaucoma has been linked to the NO-related oxidative stress due to endogenous and exogenous ROS. Studies on animal models revealed GBEs neuroprotective, anti-oxidant, and anti-inflammatory properties on RGCs.[31,129–132] Most recent clinical studies have shown that GBE increases ocular blood flow, however, the visual field (VF) impact continues to be inconclusive.[133] Given a wide potential of the active substances found in GBE in addressing glaucomatous damage on a molecular level, studies were performed to assess its effect on cases of NTG to see whether we can leverage its neuroprotective properties.

GBE AND NTG

Ocular Blood Flow

Glaucoma patients who were prescribed GBE for a short period of time of two days had enhanced ophthalmic artery end-diastolic velocity as measured by color Doppler imaging.[134] A different study showed that GBE also increases blood flow velocity in the retrobulbular vasculature, superior, and inferior capillaries compared with placebo, and reduced vascular resistance in central retinal and nasal short posterior ciliary arteries.[135] A study with NTG patients, specifically, who were prescribed GBE for 4 weeks, demonstrated increased peripapillary blood flow, and increased blood volume and velocity.[136]

Choroidal Blood Flow

Choroidal blood supply to the retina is essential in maintaining nerve fiber layer nourishment, which is impacted in NTG. Juárez et al examined GBE's effect on the choroid in rodents and discovered enlarged vessel caliber and high flow rate, which provided cells with the necessary metabolic demands.[137] More studies need to be done on humans to address both short and long-term effects of a therapeutic dose of GBE on choroidal blood flow.[138]

Trabecular Meshwork

Changes in the trabecular meshwork (TM) are involved in glaucoma as degeneration of the sclerocorneal TM layer alters porosity and reduces drainage of aqueous humor. A DNA analysis of patients with primary open angle glaucoma showed high expression of endothelial-leukocyte adhesion molecules, which results in imbalance of redox reactions.[139] Experimental models with steroid-induced changes to the TM revealed that GBE prevents dexamethasone-induced changes to the TM.[140] GBE administration suppressed steroid-induced increased IOP, reduced accumulation of extracellular materials in the cribriform layers, resulted in better cellularity of TM cells, and lowered steroid-induced myocilin expression. On a molecular level, GBE attenuated apoptosis promoted by anti-Fas ligand, modulated the expression of αβ -crystallin and heat-shock protein 70 and 90.[140] These findings indicate that GBE exhibits properties that can be extrapolated to counteract the changes that occur within the TM.

RGCs

RGCs located in the inner layer of the retina receive information from photoreceptors and transmit this information to several brain regions. Studies investigated the effect of GBE against neurotoxicity of the environment formed by the artificially induced high IOP. Hirooka et al produced elevated IOP by cauterizing three episcleral vessels, and then measuring secondary degeneration in a group receiving GBE for 5 months and a control group without anything.[141] The group receiving GBE had significantly lower levels of RGC loss in the superior colliculi brainstem region that is involved in vision processing.

Studies utilizing an optic nerve crush model showed that GBE application increased the survival rate of RGCs on a side with a crushed ONH in a dose-dependent manner.[108] As RGC loss is one of the underlying markers of glaucomatous damage, including conditions of NTG, it can be used to halt glaucoma progression.

Neuroprotection

GBE's neuroprotective properties on RGCs have been demonstrated in hypoxic conditions. Oxidative injury produces hydrogen peroxide and hypoxic injury was induced by clamping the ONH with a "microserrefine clip" with an applicator. The density of RGCs measured was higher in animals treated with EGb 761 in both vivo and vitro cases, exhibiting neuroprotective effects.[142] Additionally, Wang et al looked at the effect of GBE on

glutamate-induced toxicity and found a decrease in percentage of RGC loss.[143] GBE's components, terpenoid and flavonoids, showed neuroprotective properties in cases of necrosis and apoptosis induced by ROS, NO, and β-amyloid-induced toxicity, and further elucidated protective effects on calcium cytotoxicity.[23]

VF Changes

A study done by Lee et al with NTG patients, testing a long-term effect of GBE on VFs, showed a significant improvement in VFs that were evaluated using Humphrey Vision Field Analyzer for a period of 4 years.[144] Both the regression coefficient and mean total deviation improved when measured before and after GBE treatment, whereas the IOPs did not show a significant change across all participants.

A case report of a patient with progressive deterioration of VF and acuity despite a stable IOP controlled by medications showed marked improvement in vision acuity with long-term GBE administration.[5] The patient's vision improved from counting fingers at 1 foot occulus dexter (OD) and 20/50 occulus sinister (OS) to 20/40 OD and 20/30 OS after 30 months of 120 mg GBE and pentoxifylline addition to a daily regimen of IOP-lowering glaucoma medications.

Quaranta et al looked at GBEs effects on 27 patients with NTG that demonstrated preexisting, progressive VF deficits.[145,146] These patients received 40 mg of GBE 3 times per week. Patients showed improvements in their VF performance compared with controls. There were no changes to IOP or blood pressure; hence, these VF improvements are due to secondary effects such as increased ocular blood flow or cognitive function, which were not measured. Guo et al studied 26 NTG Chinese participants and reported that GBE had no changes in VFs.[147] However, they did not show proof of VF deterioration before the study began. Quaranta et al reported that there was no distinction made between NTG and isolated ischemic event in their sample.[145,146]

Adverse Effects

Multiple studies have reported minimal adverse effects (AEs) of GBE within a specific prescribed dosage range.[126,148,149] Overall, GBE continues to be a well-tolerated supplement with a low side effect profile. Systematic reviews of Cochrane database,[150,151] PubMed/MEDLINE, EMBASE,[151,152] and Google Scholar,[151] report no statistically significant difference in AEs between 80 and 600 mg of GBE and placebo. Several self-reported AEs included upset stomach, headache, dizziness, constipation, palpitations, and allergic skin reactions.[153]

Findings show spontaneous hyphema in patients taking GBE[154]; however, assessment of 29 coagulation parameters showed no evidence of altered blood coagulation or platelet aggregation.[155] GBE was detected to bind cytochrome P450-CYP2C9, which is responsible for the metabolism of warfarin and nonsteroidal anti-inflammatory drug flurbiprofen.[156,157] Studies reported no increase in bleeding risk nor any interaction between EGb 761 and phenprocoumon, acetyl salicylic acid, or anticoagulative, antiplatelet medication.[158]

FUTURE DIRECTIONS

Flavoprotein Fluorescence Imaging

Flavoprotein fluorescence (FPF) is a sensitive biomarker for monitoring functional retinal metabolic health at a subclinical level, providing insight to mitochondrial damage that resulted from oxidative stress.[159] Previous studies have shown that increasing oxidative stress led to gradual increases in FPF levels.[160,161] Increased FPF levels were positively correlated with both decreased mitochondrial membrane potential and increased risk of apoptosis via caspase-3.[160,161] FPF signal intensity is consistent with the proportion of flavin adenine dinucleotide molecules in oxidized electronic states.[162] Lower-energy electrons, as seen in oxidized states, are more susceptible to blue light excitation and a green autoflorescent light is emitted. It is important to note that FPF signal is only detectable in living cells and intensity correlates with the level of mitochondrial damage.[162] Given aforementioned molecular pathology of mitochondrial damage, this technology can be used as a screening tool for studying the effects of GBE by bringing scientific technologies to the patients.

Further Suggestions

Studies looking at GBE's effect on ocular blood flow parameters have relied on technology such as Color Doppler imaging (CDI) and confocal scanning laser Doppler flowmetry (CSLDF)[163]. CDI software allows looking at larger retroocular blood vessels' oxygen delivery, pH, pCO_2, potassium levels etc, but it cannot be used to visualize retinal vasculature. CSLDF, in contrast to CDI, is able to provide information on retinal vasculature, but is hard to reproduce due to high sensitivity to illumination changes and eye movement.[164] An alternative solution is to use technology such as ocular coherence tomography angiography, which is becoming greatly utilized in the field for assessing ocular blood flow. In addition, long-term prospective clinical studies with randomized controls should be performed to elucidate the effect of GBE on NTG patients. Molecular research should apply focus on emerging evidence of mitochondrial involvement in people with NTG compared with the healthy controls.

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