Current Perspectives on the Beneficial Role of *Ginkgo biloba* in Neurological and Cerebrovascular Disorders

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ABSTRACT: *Ginkgo biloba* extract is an alternative medicine available as a standardized formulation, EGb 761®, which consists of ginkgolides, bilobalide, and flavonoids. The individual constituents have varying therapeutic mechanisms that contribute to the pharmacological activity of the extract as a whole. Recent studies show anxiolytic properties of ginkgolide A, migraine with aura treatment by ginkgolide B, a reduction in ischemia-induced glutamate excitotoxicity by bilobalide, and an alternative antihypertensive property of quercetin, among others. These findings have been observed in EGb 761 as well and have led to clinical investigation into its use as a therapeutic for conditions such as cognition, dementia, cardiovascular, and cerebrovascular diseases. This review explores the therapeutic mechanisms of the individual EGb 761 constituents to explain the pharmacology as a whole and its clinical application to cardiovascular and neurological disorders, in particular ischemic stroke.

KEYWORDS: *Ginkgo biloba*, ischemic stroke, antioxidants, flavonoids, Alzheimer's disease

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Introduction

Complementary and Integrative Health (CIH) as defined by the National Institutes of Health (NIH) is a classification of diverse therapies, which includes practices and products that are generally not accepted as conventional medicine, which consists of natural, vitamin, and mineral products, as well as movement- and diet-based therapies. The most commonly used CIH products include omega-3 oils, glucosamine, and *Echinacea* to treat a wide variety of health conditions, such as musculoskeletal problems, cold, and depression. The Centers for Disease Control and Prevention estimate that the use of CIH is increasing in the United States, from 36.0% of adults in 2002 to 38.3% in 2007.[1] Despite the promise of many CIH products, they are not approved by the U.S. Food and Drug Administration (FDA), thus the products are not regulated like conventional medicines, which can lead to erratic dosing and possible safety issues.

Ginkgo biloba extract is a commonly used CIH product in the United States and is obtained from *G. biloba* trees native to China. *Ginkgo* trees have a long history of use in traditional Chinese and Japanese cooking and medicine to treat conditions such as asthma, cough, and enuresis.[2,3] Modern medicinal uses for *Ginkgo* are derived solely from leaf extracts; however, like most natural products, the location of growth, the time of extraction, and other factors can change the constituents of the product. A standardized formulation, EGb 761®, also sold as Tanakan® or Tebonin®, was created to normalize the constituents to assure reliable and consistent drug performance and the absence of ginkgolic acid, a known allergen naturally found in *Ginkgo*.[4] The standardized preparation of EGb 761 involves harvesting *Ginkgo* leaves while still green, and after morphological analysis, they are extracted in 60% (w/w) acetone and water, concentrated, and analyzed by high-performance liquid chromatography. The final product is adjusted to ~24% flavone glycosides (primarily quercetin, kaempferol, and isorhamnetin), 6% terpene lactones (consisting of 2.8%–3.4% ginkgolides A, B, and C, and 2.6%–3.2% bilobalide [BB]), and <5 ppm ginkgolic acid.

The use of EGb 761 has not yet garnered FDA approval in the United States, but it is available by prescription in European countries. There are multiple clinical trials that have investigated and are currently investigating its use in various diseases such as cardiovascular disease (CVD), hearing loss, and cognitive deficient conditions like Alzheimer's disease (AD). EGb 761 is a safe natural product for human use, as it shows minimal side effects,[5,6] no monoamine oxidase inhibition,[7] and no cytochrome P450 (CYP450) enzyme inhibition,[8] although it may cause CYP3A4 induction.[9] The therapeutic mechanisms of EGb 761® can be attributed to its individual constituents whose differing mechanisms of action may lead to a pharmacological synergy within the formulation.[10,11] This review explores the recent clinical and

preclinical discoveries and advances in the use of EGb 761 and its individual constituents with a focus on neurological, cardio-, and cerebrovascular pathologies.

Individual Components

Chemical structures for the constituents of EGb 761 are shown in Figure 1, and the formulation consists of the trilactone terpene, ginkgolides A, B, and C (ginkgolides J and M, not shown, are present in lower concentrations), and flavonoids that are present as flavonol-*O*-glycosides. Ginkgolides have been clinically shown to act as platelet-activating factor (PAF) antagonists, inhibiting platelet aggregation and promoting increased blood flow.[12] Flavonoids are known to act as antioxidants and heavy metal chelators due to their phenolic structures,[13] and they have been clinically investigated in cardiovascular[14] and inflammatory diseases.[15] Additionally, BB has shown anti-inflammatory properties and neuroprotection in preclinical models of stroke[16] and AD.[17] The standard clinical dose of EGb 761 is 120 mg (~1.7 mg/kg) once or twice daily; thus, a standard dose will contain ~3–4 mg ginkgolides A, B, and C, 3–4 mg BB, and 29 mg flavonoids. The individual constituents of EGb 761 have been separately tested, either clinically or preclinically, and their effects and underlying mechanisms help to elucidate the pharmacology of the extract as a whole.

Ginkgolide A. Ginkgolide A (GA, BN52020) is an effective antagonist of PAF, a phospholipid-derived messenger that is involved in immune response to infection[18] and neuronal damage from ischemia[19] and excitotoxic injury.[20] Inflammatory injury causes an increased production of PAF, which can act as an autocrine, paracrine, or endocrine messenger to induce inflammatory proteins by activation of PAF receptor (PAFR). The administration of 30 mg/kg GA prior to *in vivo* lipopolysaccharide insult resulted in a decrease in inflammatory mediators, such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2, and tumor necrosis factor alpha in a model of *Helicobacter pylori* infection.[18] The authors attribute these effects to PAF interference on the phosphoinositide 3-kinase (PI3K) cascade in response to lipopolysaccharide activation, which was blocked by GA.[21] In a separate study, PAF applied to neuronal cultures caused dose-dependent cell death that was ameliorated by the addition of ginkgolide or an NOS inhibitor. This supports the role of PAF in NO-mediated pathology, which can be rescued with PAF antagonists.[20] PAFR has been shown to be involved in Jak/STAT signaling pathways that are responsible for activating the transcription of various proteins in response to cytokines or growth factors. As low as 10 μM GA was able to reduce STAT3-mediated inflammatory response in vascular endothelia stimulated by high glucose.[22] This finding was confirmed in a separate study in which ginkgolide decreased STAT phosphorylation after spinal cord injury in rats, resulting in reduced apoptosis and improved outcome.[23] Although not the most potent PAF antagonist, GA was found to have antioxidant capacities as shown by free-radical spin trapping in an animal model of ischemia.[24] For these reasons, Weakley et al incorporated GA into a gold nanoparticle (GA-GNP) for use in the reduction of neointimal hyperplasia after arterial reconstruction.[25] A 10 μM concentration of GA-GNP was able to achieve a sustained exposure equivalent to 50 μM GA. The nanoparticles were shown to inhibit smooth muscle cell migration by reducing Extracellular-signal-regulated kinases 1/2 (ERK1/2) activation and superoxide formation. The mitogen-activated protein kinases (MAPKs) such as ERK1/2 are kinases that are involved in the regulation of cell proliferation in response to various stimuli, and the decreased phosphorylation of ERK1/2 by GA is attributed to the decreased proliferation of arterial smooth muscle cells.

Unique to GA is its anxiolytic property, which is not observed in any other constituent of EGb 761.[26] Kuribara et al noted anxiolytic effects of GA at a far lower dose (1–2 mg/kg) than EGb 761 (125 mg/kg), which correlates with the standardized concentrations of GA in EGb 761 (~1%–2%). These effects were not blocked by the GABA antagonist flumazenil, and higher doses of GA were safer than EGb 761, which caused suppressed avoidance and reduced caffeine stimulation. Conventional anxiolytics work through the

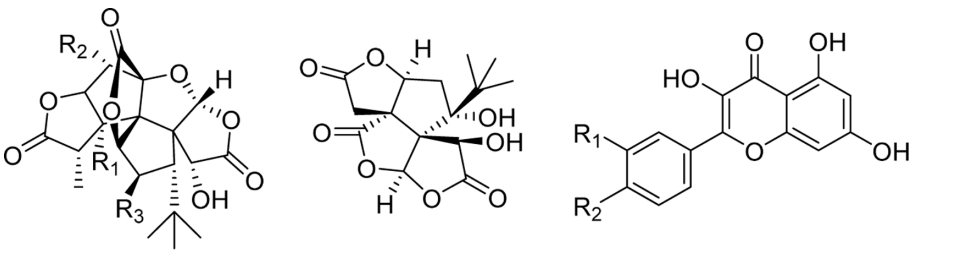

Figure 1. Structures of EGb 761 constituents.

$GABA_A$ receptor to give an inhibitory response and also result in sedation and other adverse side effects. Because the anxiolytic effect of GA was not blocked by GABA antagonism or adenosine receptor stimulation by caffeine, this suggests a novel mechanism. Further investigation by Oliveira et al revealed increased cAMP-responsive element binding protein (CREB) levels in the dorsal hippocampus and amygdala regions of EGb 761-treated rats that may account for the anxiolytic effects of EGb.[27]

Ginkgolide B. Ginkgolide B (GB, BN52021) is the most potent PAF antagonist of the EGb 761 constituents and has been clinically tested for efficacy in sepsis,[28–30] multiple sclerosis,[31] migraine,[32] and ischemia/reperfusion (I/R) injury following transplantation.[33] In a phase III, double-blind clinical trial, 120 mg BN52021 was shown to be safe and effective at treating patients with severe gram-negative sepsis, which was attributed to its PAF antagonist properties.[30] Patients treated with antimicrobials plus GB showed a significant drop in mortality as compared to patients treated with antimicrobials and placebo; this finding led to the development of other PAF antagonists for the treatment of sepsis.[30] Administration of 2–10 mg/kg BN52021 to patients who underwent lung transplantation was able to optimize pulmonary function in a randomized trial, which was attributed to lower postoperative PAF concentrations.[33] The necessary ischemia and subsequent reperfusion of transplanted tissue causes injury that is amplified by PAF, which can cause neutrophil activation and cytokine release.[34] Recently, GB was also shown to reduce cerebral I/R injury in an animal model, which was ascribed to decrease the expression of cathepsins B and L, lysosomal proteases implicated in ischemic cell death.[35] As the chief component in Migrasoll® (60 mg GB with coenzyme Q10, riboflavin, and magnesium), GB was shown to be clinically effective in the treatment of migraine with aura. The administration of Migrasoll® at the onset of the subjects' second migraine reduced both the time of aura and the severity of the migraine with respect to the subjects' prior episode.[32,36,37] The underlying cause for migraine is thought to be due, at least in part, to hyperexcitability of neurons, possibly by K^+ or glutamate-dependent mechanisms.[38] This hypothesis is supported by the improvement of symptoms after administration of lamotrigine,[39] a Na^+ channel and voltage-gated Ca^{2+} channel blocker that inhibits the release of glutamate. Presynaptic PAFR activation has been shown to increase glutamate release through a mechanism that is not well understood. The ability of GB to inhibit PAF and its influence on glutamate release[20] may explain the mechanism behind the reduction of migraine severity with GB and Migrasoll®.

Ginkgolide C. Ginkgolide C (GC) is a less-studied constituent of EGb 761 that has OH substitutions on each of the R-positions, which affect its affinity and stability. When compared to GB, GC was found to be a roughly 25-fold less-potent PAF antagonist.[40] This is attributed to the presence of the 7β-OH substitution not present on the other ginkgolides. Indeed, it has been shown that GC has lower AUC and C_{Max} than the other ginkgolides[41] and undergoes *in vivo* methylation much more rapidly that the other EGb 761 constituents,[42] which may account for the overall reduced activity. Even so, GC was shown to inhibit *in vitro* platelet aggregation at 100 μM through stimulation of matrix-metalloproteinase-9,[43] which has been shown for the other EGb ginkgolides[44] and suggests a similar mechanism. Thus, while GC may not be bioavailable at clinically relevant concentrations due to rapid metabolism, it may contribute to the overall pharmacology of EGb 761.

Bilobalide. BB is a trilactone terpene that accounts for ~2.9% of the standardized EGb 761, yet, while structurally similar to the ginkgolides, it is not a direct PAF antagonist. Recently, however, a study by Maerz et al shows a dose-dependent down-regulation of PAFR mRNA in the presence of BB (10 mg/kg) in an *in vivo* model of myocardial infarction.[45] BB treatment reversed the upregulation of PAFR seen in the untreated cardiac tissue while levels of the protein responsible for PAF metabolism, PAF-acetylhydrolase, remained unchanged, leading to an overall reduction in PAF signaling. The reduction of PAFR levels due to BB and the PAF antagonist properties of the ginkgolides may lead to a synergistic effect, and thus further decreasing PAF signaling upon administration of EGb 761 as a whole.

Similar to the ginkgolides, BB has shown other beneficial properties in addition to its effect on PAF. Recent studies support the anti-inflammatory properties of BB through reduction of neuronal inflammation after I/R injury,[16] hypoxia-induced inflammation,[46] and inflammatory pain.[47] Additionally, Lang et al showed strong *in vivo* neuroprotection of BB (10 mg/kg) attributed to a marked decrease in glutamate release in the infarct and penumbra areas of rats subjected to permanent ischemic stroke.[48] A 10-fold increase in glutamate release was observed in control animals subjected to stroke but was "strongly attenuated" by pretreatment with BB, which resulted in reduced excitotoxicity and smaller infarct volume. The effect of BB on postischemic glutamate levels was further shown in a subsequent study with aged animals[49] and suggests that BB may provide protection from neurodegenerative diseases as well. Complementary to its neuroprotective properties, BB was recently shown to induce neuronal differentiation of P19 cells in a time-dependent and concentration-dependent manner via the Wnt/β-catenin signaling pathway.[50] The canonical Wnt/β-catenin pathway induces cell proliferation by the translocation of β-catenin to the nucleus after Wnt binds to its receptor. Activation of the Wnt pathway by GSK-3β phosphorylation was observed after BB treatment, and abolished upon application of a Wnt inhibitor, which suggests that BB may facilitate neuroregeneration. For diseases and conditions causing neuronal damage, such as stroke or AD, therapies that can initiate or increase neuroregeneration can contribute to a multifaceted and potentially more effective treatment.

Flavonoids. The terpene-free fraction of EGb 761 consists primarily of flavonoids (CP 205) and polyphenolic

molecules that exist in the EGb 761 formulation primarily conjugated at the 3- or 7-position with D-glucose, L-rhamnose, or glucorhamnose.[2] Flavonoids are known to modulate or directly act on various cell signaling pathways, such as Akt/protein kinase B (PKB), PI3K, MAPK, and protein kinase C cascades. Extensive and useful reviews cover their involvement as signaling molecules,[51] in brain function,[52] cognition,[53] and cell survival.[54] The most studied aspect of flavonoids is their ability to act as antioxidants by directly scavenging oxidants and free radicals, chiefly hydroxyl radicals, and by delocalizing the resulting unpaired electron within the polyphenolic structure. This property has led to their use in oxidative and inflammatory pathophysiologies such as AD,[53] cancer,[55] and heart disease.[56]

One particular flavonoid in EGb 761, quercetin, has been clinically investigated for its effect on hypertension. Acute oral administration of quercetin aglycone lowered blood pressure in hypertensive males by a mechanism that was independent of angiotensin-converting enzyme (ACE) activity, endothelin-1, or NO bioavailability.[57] These effects, however, were not observed with a lower (160 mg) daily administration of quercetin.[14] The lowered blood pressure from an acute high dose (1095 mg) may be due to rapid scavenging of superoxide and H_2O_2, which are involved in vasoconstriction.[58] Other clinical studies using daily administration of quercetin showed mixed results regarding vascular function,[15] inflammation,[59,60] and infection.[61,62] Beyond oxidant scavenging, the flavonoid isorhamnetin was able to upregulate antioxidant enzymes through Nrf2 activation.[63] One enzyme upregulated by isorhamnetin, heme oxygenase 1 (HO1), has been identified by our group and others as a key element to the neuroprotection afforded by EGb 761 in stroke models.[64,65] HO1 is an antioxidant enzyme that catalyzes the degradation of free heme to bilirubin and carbon monoxide, which is a vasodilator at low concentrations. Another flavonoid in EGb 761, kaempferol, was recently shown to have anticoagulant properties partially attributed to PI3K/Akt/PKB signaling,[66] which may complement the similar processes of other EGb 761 constituents.

EGb 761 as a Therapeutic

EGb 761 in clinical trials. At the time of access, EGb 761 was listed as an intervention in 51 ongoing and completed clinical trials according to the NIH (Clinicaltrials.gov, accessed 9/2015) for the treatment of 17 conditions at sites spanning 14 countries. The disease conditions targeted by EGb 761 intervention are listed in Table 1, which shows that the applications of EGb 761 tend to be neurological (26), eye/ear (10), or cardio/cerebrovascular (5) in nature. Of the trials listed, 12 studies specifically list EGb 761 as an intervention, and 16 trials remain open. Geographically, most EGb clinical trials are performed in North America (18) and east Asia (17), followed by Europe (9). A literature search of Pubmed.gov indicated that over 25% of research articles pertaining to EGb 761 have been published in the past five years, and 50% of all such articles published in the past 10 years. Together this demonstrates the recent popularity of EGb 761, despite knowledge of its therapeutic properties for ages.

Table 1. Disease conditions of active and completed clinical trials involving EGb 761 as an intervention.

CONDITION	TOTAL TRIALS	ACTIVE TRIALS
Cognition and memory	18	3
(Alzheimer's and dementia)	(4)[a]	–
Hearing loss	5	3
Ocular disease	5	2
Cardiovascular disease	4	3
Drug interaction and drug metabolism	3	–
Asthma	2	–
Schizophrenia	2	–
Stroke	2	1
Type-II diabetes mellitus	2	–
Vitiligo	2	1
ADHD	1	1
Brain injury	1	–
Depression	1	1
Hypoactive sexual desire disorder	1	–
Intrauterine growth restriction	1	–
Multiple sclerosis	1	–
Obesity	1	1

Notes: Data taken from Clinicaltrials.gov accessed 9/2015. [a]Trials are a subset of cognition and memory.

Cognition, memory, and dementia. A large majority of clinical trials involving EGb 761 are directed at the improvement of cognition and memory, some of which target dementia,[6] and more specifically AD.[67] A recent study of the effect of EGb 761 on memory in healthy, middle-aged subjects indicated that, when administered daily, EGb 761 (240 mg daily) may improve some aspects of memory better than others.[68] The study, performed in Germany, found that EGb-treated subjects significantly improved in a memory recall test after a six-week regimen. Other studies in healthy individuals suggest that improvement of cognition, memory, or self-estimated mental health were attributed to EGb 761.[69–72] There is, therefore, great interest in the effect of EGb 761 on cognitive impairment and memory loss due to disease and aging.

Dementia is a category of brain diseases characterized by a gradual decline in cognition and memory that includes AD, vascular dementia, Lewy body dementia, and frontotemporal dementia.[6] The abilities of EGb 761 to modulate excitotoxic glutamatergic neurotransmission,[73] reduce amyloid-β aggregation and toxicity,[74] and function as a radical scavenger[75] suggest its use in the various dementia pathologies. Clinical studies of 240 mg daily EGb 761 administration to patients with dementia indicate its efficacy in stabilizing or slowing

the decline of mental function, particularly for patients with neuropsychiatric symptoms.[5,67,76] The European studies included AD patients with Neuropsychiatric Inventory (NPI) composite scores >4 and reported significant improvements in NPI, as well as reductions in depression and anxiety. Additionally, a clinical investigation into the use of EGb 761 with a commonly prescribed cholinesterase inhibitor, donepezil, suggests that the combination of the two therapies is more effective than either one alone.[77] It was shown that EGb 761 was not effective in preventing dementia[78]; however, the clinical evidence for the use of EGb 761 to slow its progression is promising and warrants further clinical investigation.

Cardiovascular disease. The efficacy for EGb as an intervention on various aspects of CVD has been investigated, with mixed results. Preclinical studies for the treatment of chronic CVDs, such as hypertension, peripheral artery disease (PAD), and Raynaud's disease, suggest that EGb 761 may provide an alternative mechanism to conventional therapies, as it was observed that 180 mg/kg EGb 761 in rats lowered blood pressure through ACE activity inhibition while regulating NO levels and preserving vascular reactivity toward endothelium-dependent and -independent vasodilators.[79] Clinical trials conducted thus far investigating the effect of EGb 761 on hypertension have found no benefit[80] or are inconclusive,[81] although it should be noted that many trials have been inadequately designed (see Ref. 81 for meta-analysis).

Raynaud's disease is characterized by acute reductions in blood flow due to vasoconstrictions in peripheral blood vessels in response to stress and is treated by Ca^{2+} channel blockers or angiotensin receptor blockers with limited efficacy.[82] Despite the multivalent actions of EGb 761 to improve circulation, multiple clinical trials failed to show its efficacy in the treatment of Raynaud's disease compared to conventional therapy[83] or placebo.[84,85] The permanent narrowing of peripheral arteries is referred to as PAD and also results in reduced blood flow to the extremities causing claudication, or pain while walking. Initial trials showed significant improvements in both pain-free walking distance[86] and area of ischemia postexercise[87] in PAD patients undergoing treatment with 300–320 mg/day EGb 761. Recent studies are less assuring, as many indicate insignificant improvement[88] or insufficient evidence.[89] The clinical data for the use of EGb 761 in CVD is thus far inconclusive; however, meta-analyses call for further investigation with improved study design.[81,89]

Ischemic stroke. The aforementioned beneficial effects of EGb 761 and its constituents on blood flow, ischemia, and neuroprotection point to its potential as a therapeutic for the treatment of cerebrovascular stroke. Investigations into the use of EGb 761 as a prophylactic therapy for ischemic stroke do not suggest that daily intake is able to prevent stroke or other cardio/cerebrovascular events.[90–92] The small trial conducted by Gardner et al measured the effect of 300 mg/day EGb 761 on platelet function and aggregation in patients already taking aspirin and found no statistically detectable impact.[90] Alternatively, promising results have been observed that support administration of EGb 761 soon after the onset of ischemia and during the recovery period.

Until recently, only minor clinical trials were conducted to investigate the effect of EGb 761 on acute ischemic stroke, and while together they suggest improvement among treatment groups compared to controls, a Cochrane Collaboration meta-analysis determined that high-quality, large-scale randomized trials are necessary to confirm such results.[93] In 2013, one such study conducted in Iran, a double-blind, placebo-controlled, randomized trial, reported protective effects of 120 mg/day EGb 761 in acute ischemic stroke.[94] The trial consisted of 102 patients and reported that 17 EGb 761-treated patients compared to 5 placebo met the primary outcome of a 50% reduction in the National Institutes of Health Stroke Scale score after four months ($P < 0.05$). Further investigations through large-scale clinical trials are warranted to support the use of EGb 761 in stroke, and additional research is needed to confirm its therapeutic mechanisms.

Preclinical research on ischemic stroke. Preclinical research that supports the use of EGb 761 for ischemic stroke describes its therapeutic mechanism as a combination of three elements: increased blood flow, neuroprotection, and neuroregeneration. There are numerous models of ischemic stroke utilized by researchers, which include *in vitro* nutrient deprivation models, such as oxygen–glucose deprivation,[95] and *in vivo* models (Table 2), which permanently or temporarily stop cerebral blood flow. Initial investigations recognized a dose-dependent increase in cerebral blood flow and decrease in glucose uptake in the presence of EGb 761,[96,97] which is protective in ischemia. This effect is similar to that observed with quercetin or BB alone and was attributed to be via Ca^{2+}/NO signaling regulation.[98,99] EGb 761-treated animals subjected to stroke are found to have increased cerebral blood flow compared to controls, which is accompanied by a reduction in infarct volume.[100,101] While restoration of blood flow after stroke elicits reperfusion injury through oxidative burst, it is necessary to recover cellular function in the penumbra of the ischemic injury; thus, the effect of EGb on cerebral blood flow contributes to its protective effects.

Table 2. EGb 761 in *in vivo* ischemic stroke models.

ANIMAL	STROKE MODEL	REFERENCES
Rat	pMCAO[a]	101,117,121–126
	tMCAO[b]	101,104,116,127
	4-VO[c]	128–130
Mouse	pMCAO[a]	65,131,132
	tMCAO[b]	105,106,133
	BCCAO[d]	134
Gerbil	BCCAO[d]	135–137

Notes: [a]Permanent middle cerebral artery occlusion; models of ischemic injury. [b]Transient middle cerebral artery occlusion; models of I/R injury. [c]Four-vessel occlusion; models of forebrain I/R injury. [d]Bilateral common carotid artery occlusion; models of global ischemia.

The most studied aspect of EGb 761 with regard to ischemic stroke is its neuroprotective effects, which can be attributed to its attenuation of excitotoxicity,[102] free-radical scavenging,[10,103] and induction of antioxidant enzymes. Evidence of EGb 761 neuroprotection has been shown through reduced infarct volume[104] and apoptosis,[105] and improved neurological function.[106] The reduction in excitotoxic injury in EGb 761-treated animals subjected to stroke is due to the suppression of ischemia-induced glutamate release,[107] which protects against neuronal death,[102] and may be attributable to the GA and BB constituents as previously discussed. There is substantial evidence that EGb 761 is able to induce the expression of antioxidant enzymes, in particular HO1,[108] through Nrf2 activation.[109,110] Knockout studies by our group depict the importance of HO1 in EGb 761 neuroprotection, as animals deficient in HO1 effectively lose the protective effect of EGb 761.[65]

After cerebral injuries such as ischemia, there is an induction of neural stem cell formation and migration to the site of injury.[111] These processes are shown to be influenced by various pro-survival signaling pathways such as Wnt/β-catenin,[112] Akt,[113] brain-derived neurotrophic factor,[114] and vascular endothelial growth factor.[115] EGb 761 has been shown to positively influence each of these pathways *in vivo* upon induction of experimental stroke,[65,116,117] which suggests that part of its beneficial effects may be due to accelerated neuronal recovery after ischemic injury. Taken together, preclinical studies propose a multivalent therapeutic effect of EGb 761 in the treatment of ischemic stroke.

Integration with Conventional Medicine

As previously discussed, EGb 761 shows minimal toxicity and is very well tolerated, even in aged patients taking multiple medications, for doses up to 240 mg/day. This profile is ideal for using EGb 761 as an adjunct or combination therapy. Due to its inhibitory effects on platelet aggregation, a small clinical trial into the combination of ticlopidine, a thienopyridine anti-platelet medication, with low-dose EGb 761 (80 mg/day) was conducted. ADP-induced platelet aggregation was found to be significantly lower in ticlopidine + EGb 761 treatment than ticlopidine or clopidogrel alone, but no effect was observed for arachidonic acid- or thrombin-induced aggregation.[92] Caution should be taken, however, when combining EGb 761 with medicines metabolized by CYP3A4, as EGb 761 may induce expression of the gene. This is particularly important for susceptible patients, such as those diagnosed with human immunodeficiency virus. It was found that EGb 761 may cause a virological breakthrough in patients taking efavirenz, an antiretroviral that is metabolized by CYP3A4, after a patient developed a virological failure while taking EGb 761, which caused a drop in plasma concentration of efavirenz.[118,119] Despite the safe profile of EGb 761, it is recommended to talk to a physician before taking any herbal supplements for possible drug–herb interactions.[120]

Future Directions

EGb 761 is a widely used CIH available as a standardized extract, which allows for consistent results and reliable dosing for the treatment of a wide range of conditions, particularly in cognitive, cardio-, and cerebrovascular diseases. More rigorous clinical trials are required to translate the promising preclinical findings to definitive and reliable clinical data. The common dose of EGb 761 or its individual constituents were found to be consistently higher in preclinical studies as compared to that of clinical trials (100 mg/kg compared to <2 mg/kg, respectively). Some clinical trials have used doses exceeding 300 mg daily, while other trials have used substantially lower doses. While the individual constituents of EGb 761 are standardized, it appears the dose is not, which makes interpreting the clinical effects of EGb 761 difficult. As outlined in this review, the individual constituents of EGb 761 each possess therapeutic qualities; thus, future studies may modify EGb 761 to increase or decrease particular constituents for a desired activity.

Author Contributions

Analyzed the data: KMN. Wrote the first draft of the manuscript: KMN. Contributed to the writing of the manuscript: ZAS. Agree with manuscript results and conclusions: KMN, ZAS. Jointly developed the structure and arguments for the paper: KMN, ZAS. Made critical revisions and approved final version: ZAS. Both authors reviewed and approved of the final manuscript.

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