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Ginkgo biloba Extract: Review of CNS Effects

Laura L. Boles Ponto, PhD[1,3] and Susan K. Schultz, MD[2]

The marketing of *Ginkgo biloba* extract (GBE) products is directed to the healthy, not diseased, population primarily for the promotion and maintenance of optimal brain function, not the treatment or prevention of any specific pathological state. However, recommendations are available for the use of GBE for a myriad of diseases that generally fall into one of three categories—cerebrovascular, peripheral vascular, or mitigation of tissue damage. The evidence for the pharmacological actions of GBE stem from one of three types of investigations—clinical studies in humans, pharmacological trials in animals, and in vitro studies. The purpose of this paper is to review the scientific literature on the central nervous system effects of GBE, with emphasis on the potential mechanisms of action. Limitations of the current scientific literature are highlighted and suggestions for future human and animal research directions are proposed.

KEY WORDS: *Ginkgo biloba* extract; EGb 761; aging.

INTRODUCTION

Significance of Use

According to a recent survey of medication use in the United States (1), 14% of respondents used a herbal/dietary supplement within the previous week with 2.2% of those surveyed using *Ginkgo biloba*. This finding extrapolates to 4.6 million GBE users within the United States. Selective age groups exhibit differing usage patterns. For example, less than 1% in the 18–44 year age group reported use of GBE, whereas, 4% of males and females in the 45–64 year age group reported use of GBE in the previous week. The highest use pattern was in ≥65-year-old women at 5%. In comparing these usage rates to the 40 most commonly used prescription and OTC drugs, GBE usage is equivalent to that of the 20th most commonly used "drug," ranitidine HCl (Zantac®) (1). Therefore, significant numbers of people are routinely exposed to the effects of GBE, justifying the need for a scientifically-based understanding of the pharmacology.

Marketing Claims of GBE

Since dietary supplements in the United States do not need to conform to the labeling requirements for drugs, the medicolegal concept of "indications" does not strictly apply. However, marketing claims (accompanied by the disclaimer statements: *These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.*) guide the consumer in the use of herbal products in much the same manner as "indications" serve to guide the use of OTC and prescription drugs by consumers and health care professionals, respectively.

GBE is currently marketed for enhancing brain function using the following language[4]:

[1]Department of Radiology, Positron Emission Tomography Imaging Center, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa.
[2]Department of Psychiatry, Positron Emission Tomography Imaging Center, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa.
[3]To whom correspondence should be addressed at PET Imaging Center, 0911 Z JPP, University of Iowa Hospitals and Clinics, 200 Hawkins Drive, Iowa City, Iowa 52242; e-mail: laura-ponto@uiowa.edu.

[4]Information compiled from Quanterra™Mental Sharpness, http://www.herbalsupps.com/quanterra_mntlshrpns.shtml and package labelling and PhysioLogics Catalog of Products, page 29.

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- Promotion and maintenance of mental sharpness, concentration, and focus

  Restorative effects on the brain—specifically memory function and the ability to concentrate. Enhances nootropic activities—agents known to enhance mental clarity, memory, and alertness

- Enhances blood circulation

  Promotes blood circulation and oxygen supply to the brain and extremities. Increases the amount of oxygen in the blood supply to the brain

- Promotes the elasticity and strengthening of blood vessels and capillary walls

  Increases vascular tone by supporting healthy circulation in the arteries

- Increases the uptake of glucose across the blood–brain barrier
- Powerful antioxidant that protects brain cells and the cardiovascular system

  Potent antioxidant that traps, neutralizes and eliminates free radicals before they damage healthy cells. Maintains healthy levels of platelet aggregating factor (PAF)

The marketing of GBE products is directed to the healthy, not diseased, population primarily for the promotion and maintenance of optimal brain function, not the treatment or prevention of any specific pathological state. However, the general impression, whether scientifically valid in characterizing the pathogenesis of age-related cognitive decline and dementia or not, is that by increasing or maintaining cerebral perfusion and glucose metabolism the risk of developing the dreaded "Alzheimer's disease" (in its lay definition) can be minimized or delayed. Therefore, the evidence for these CNS claims for the healthy population will be examined in this paper.

Characteristics of GBE

The Ginkgo tree is the last living species of the Ginkgoaceae family of trees, dating back 200 million years. Although the Ginkgo tree died out in Europe during the Ice Age, this one species survived in China. Individual trees have been known to live for hundreds of years reaching heights of 30–40 m and diameters of 4 m (2). Illustrative of the resilience of this plant species, the first green growth in the center of Hiroshima after the atomic bomb explosion was a sprout of a Ginkgo tree. Today, this tree lines the streets of our busiest cities as a hardy ornamental. The resistance of this "living fossil" to mutagenic insults as well as to modern day environmental toxins has sparked an interest in the biologic and pharmacologic potential of the antioxidant and free-radical scavenger abilities of components of Ginkgo leaf extracts.

The leaves, fruits, and seeds of this tree have been a part of Chinese herbal medicine for the treatment of bronchial asthma, as a wound dressing and a memory enhancer since 2800 B.C. (3, 4). However, interest by Western medicine in the utility of the leaves of the Ginkgo tree has been limited to the latter half of the twentieth century. In the 1950s, the Dr Willmar Schwabe phytopharmaceutical company of Germany developed a concentrated, standardized form of *Ginkgo biloba* extract (GBE) referred to as EGb 761. This product has 24% flavonoids (including monosides, biosides, and triosides of quercetin, isorhamnetins, 3′-*O*-methylmyristicins, and kaempferol) and 6% terpenes (including the diterpenes ginkgolide A, B, and C and the sesquiterpene bilabolide) with less than 5 parts per million of ginkgolic acids (2–4). The bioflavonoids are believed to be responsible for the antioxidant activities (5) while the terpene lactones are believed to promote circulation. The structures and general antioxidant activities of 29 compounds isolated from GBE have been characterized (6). Recently, the pharmacological actions of specific components, e.g. bilabolide (7–9), ginkolide B (10), kaempferol (11), have been studied, however, the bulk of the scientific research on the therapeutic value of GBE has utilized the standardized extract, EGb 761 (12, 13). Whether the response to GBE is due to specific, isolatable components or the synergy of the entire phytopharmaceutical is currently a subject of on-going investigation (5, 14). However, many researchers believe that the combination of constituents present in EGb 761 synergistically contribute to the overall effect on cognition (15, 16).

Clinical Indications for GBE

The Federal Health Agency of Germany through its Commission E has established three primary clinical indications for GBE (4, 17):

- The symptomatic treatment of cognitive disorders generally referred to as "cerebral insufficiency"

- The improvement in pain-free walking distance in peripheral arterial occlusive disease (i.e., intermittent claudication)
- The treatment of vertigo and tinnitus of vascular and involutional origin

The fundamental mechanism of action attributed to the utility of GBE for the above indications is the induction of arterial and venous vasoactive changes that increase tissue perfusion and peripheral and cerebral blood flow (18). In a broader sense, recommendations are available for the use of GBE for a myriad of diseases that generally fall into one of three categories—cerebrovascular, peripheral vascular, or mitigation of tissue damage with the evidence for the pharmacological actions of GBE stemming from one of three types of investigations—clinical studies in humans, pharmacological trials in animals, and in vitro studies.

GENERAL THERAPEUTIC AND ADVERSE EFFECTS

In 1992, Kleijnen and Knipschild (19) systematically reviewed the human studies investigating the utility of GBE for "cerebral insufficiency" and intermittent claudication. They concluded that GBE had a low risk for side effects and that GBE therapy was warranted for patients with mild to moderate cerebral insufficiency. Chavez and Chavez (20) updated and expanded this review to include not only trials for dementia and peripheral vascular insufficiency but also a number of other diseases including asthma, cochlear deafness, depression, chronic active hepatitis B, hyperlipidemia, erectile dysfunction, cyclic edema, diabetic retinopathy, premenstrual syndrome, senile macular degeneration, shock, tinnitis, and vertigo. In reviewing more than 70 clinical trials, the adverse effects reported were few, occurring in less than 1% of patients, and generally mild in severity. Gastrointestinal disturbances, headache and dermal hypersensitivity were the most frequently reported side effects.

Three cases of unexplained bleeding, two subdural hematomas and one right eye hyphema, in patients taking GBE have been reported by Chavez and Chavez (20). Vaes and Chyka (21) identified two additional subjects with bleeding abnormalities potentially attributable to GBE. Unusual bleeding has been attributed to the inhibition of platelet-activating factor (PAF) by the GBE component, ginkgolide B, and has led to cautions regarding the concomitant use with other drugs with antiplatelet activity such as aspirin and NSAIDS and with anticoagulants (22, 23).

McKenna *et al.* (12) systematically examined the efficacy, safety and use of GBE in clinical and preclinical applications. Reviewed preclinical studies have examined the evidence for GBE effects on cardiovascular and circulatory functions, peripheral vascular function, thrombosis, hemostatis and embolism, neurodegenerative disorders, retinopathies, cognitive functioning and memory, anxiety and stress responses, receptor and neurotransmitter-mediated functions, antiproliferative activitity, and antioxidant activities. Reviewed clinical studies have examined the evidence for GBE effects on cardiovascular dysfunctions, intermittent claudication, ulcerative colitis, anti-inflammatory and antioxidant activities, acute mountain sickness, Alzheimer's disease and dementias, age-associated cognitive decline, retinopathies, memory function in healthy volunteers, depression, psychoses, erectile dysfunction, premenstrual syndrome, and exercise-induced asthma. In addition, they reviewed an extensive number of case reports involving the safety of GBE.

Maclennan *et al.* (24) reviewed the human, animal and in vivo/in vitro literature on the CNS effects of GBE and ginkgolide B. Gohil (25) focused his review on the genomic responses of herbal extracts using GBE as the prototypical example.

SCIENTIFIC EVIDENCE FOR GBE CNS EFFECTS

Healthy Subjects

A number of studies have examined the effects of GBE on cognitive functioning in nonpathological populations with mixed results. Itil *et al.* (26) examined the acute effects at 1, 2, 3, 5, and 7 h post-administration of doses of placebo, 40, 120, and 240 mg of EGb 761 on quantitative EEG measures (CEEG) in healthy males (age 18–65 years). Dynamic brain mapping of 14 brain areas found increases in the 7.5–13.0 Hz alpha activity for all doses with the 120 and 240 mg doses significantly different from placebo. These same authors applied a similar methodological approach using EEG measures to assess the bioavailability of various Ginkgo products (27). Rigney *et al.* (28) investigated the effects on cognition of short-term GBE (2 days) treatment with various dosing schemes versus placebo. The results demonstrated that the effects were more pronounced for memory

function, particularly working memory, than for other aspects of cognition and more apparent for individuals aged 50–59 years. Older studies examining the effect of GBE for age-related memory impairment in elderly subjects (29) and memory function in healthy female volunteers (mean age = 32) (30, 31) used single oral doses between 120 and 600 mg administered 1 h prior to testing.

Kennedy *et al.* (32) examined the effects of 120, 240, and 360 mg of a standardized extract of Ginkgo (GK 501 = 24% flavone glycosides, 6% terpene lactones) versus placebo using a multidose, double-blind, balanced, cross-over design in healthy, young volunteers (age 19–24 years). Evaluations were made using the Cognitive Drug Research (CDR) computerized assessment battery at 1, 2.5, 4, and 6 h post oral administration. GBE produced a number of significant time- and dose-specific changes in cognitive performance. The authors highlighted the dose-dependent improvement in "'speed of attention" with both the 240 and 360 mg doses that was evident at 2.5 h and still evident at the 6 h testing. This same group extended their research to the evaluation of the combination of GBE and *Panax ginseng* as a single dose (33) and 12 week dosing in middle aged (38–66 years) volunteers (34). The combination demonstrated a dose-dependent improvement in the "quality of memory" and a decrement in the "speed of attention" factor at the higher doses on the single dose study in young volunteers and improvements in the Index of Memory Quality (average improvement of 7.5%) for the multidose study in older volunteers. The memory enhancing effects were seen throughout the 12-week dosing period as well as during the 2-week washout period.

Mix and Crews (35) examined the effects of 6 weeks of therapy with EGb 761 at 180 mg/day in cognitively intact older subjects (55–86 years). Significant improvement was found in speed of processing abilities (Stroop Color and Word Test color naming test) with trends toward significance on three of four other tasks involving a timed, speed of processing component. However, no significant differences were found between EGb 761 and placebo-treated groups on objective memory measures. Yet, more participants in the EGb 761-treated group rated their overall abilities to remember "improved" by the end of the trial compared to the placebo group. The authors concluded that the combination of the objective and subjective measures may indicate the potential efficacy of short-term EGb 761 in enhancing certain neurocognitive functions/processes in cognitively intact older adults. Furthermore, the lack of significance on some objective measures may be limitations of the standardized tests to characterize the subtle memory-enhancing potential of EGb 761 that was detected by the subjects themselves.

Stough *et al.* (36) examined the neuropsychological changes in healthy participants (18–40 years of age) of 30 days of *Ginkgo biloba* Forte (24% flavonal glycosides, 6% terpene substances) at 120 mg/day. Two broad findings were reported: 1) GBE improved memory, particularly working memory and memory consolidation, and 2) the improvement in functioning was evident to the participants even though the study was double-blinded. In contrast, further work by this same research group found no acute nootropic effects in healthy, older subjects (mean = 58.5 ± 10.9 years, range = 50–70 years) of a single 120 mg Ginkgo-forte dose. The study employed a repeated measures, double-blind, placebo-controlled design in which all subjects were tested at 90 min postoral dosing (peak GBE plasma level) after both the placebo and the GBE, separated by a 7-day wash-out period. "Normal healthy" was defined by a self-reported lack of a history of dementia, psychiatric or neurological disorders, not by formal testing procedures. The authors commented on the inconsistencies within the limited amount of research, highlighting the importance of subject group (i.e., old versus young), dose, testing time frame and acute/chronic effects.

Moulton *et al.* (37) examined the effect on memory of BioGinkgo (LI 1370 = 27% flavonoid glycosides, 7% terpene lactones) 120 mg/day × 5 days in healthy male college students (20.6 ± 1.9 and 20.4 ± 1.8 years for GBE and placebo, respectively). No significant differences existed on any tests between the GBE and placebo groups except for the Sternberg Memory Scanning Test. However, in order to avoid practice effects, baseline measures were not taken and all comparisons were made posttreatment with the comparability of the two groups assumed. In addition, the authors acknowledged the potential for "ceiling effects" with this group, the short duration and relatively low dosage employed.

A recent report on the utility of GBE for memory enhancement in healthy older subjects (38) received a substantial amount of lay press. In this study, 230 community-dwelling, volunteers older than 60 years and MMSE > 26 were evaluated in a 6-week randomized, double-blind, placebo-controlled, parallel-group trial of GBE (Ginkoba®, Boehringer Ingelheim Pharmaceuticals), 40 mg TID. Each subject was evaluated on 14 standard neuropsychological tests, a self-reported memory questionnaire and

a global evaluation completed by a spouse, relative or friend. No significant differences were found on any measure, questionnaire or evaluation. Although side effects were not specifically monitored, no subject spontaneously reported a side effect or discontinued treatment due to an adverse effect. This study was designed to mimic the manufacturer's recommendation for GBE treatment—specifically, a dose of 120 mg/day—with an evaluation at 6 weeks, 2 weeks longer than the 4-week interval by which noticeable effects are claimed to be evident. Acknowledging the potential for ceiling effects and the lack of content analysis of the GBE product used, the authors concluded that their research did not support the manufacturer's claims of the benefits for Ginkgo on learning and memory in healthy individuals.

From this review, it is evident that the literature contains radically conflicting reports on the utility of GBE in healthy subjects. Specific issues related to methodology and therefore, comparability, of the studies include the analytical specifications of the GBE product used, the dosage administered, the duration of the investigation, specific types of cognitive/memory testing employed, and the use of baseline measurements. In all cases the outcome measure of interest was a change or a difference from placebo in cognitive/memory performance. None of the studies directly measured changes in cerebral blood flow, cerebral metabolism, or cerebrovascular tone the purported CNS mechanisms of action.

Cerebral Insufficiency

The efficacy of GBE for Alzheimer's and vascular dementias has been studied in more than 40 clinical trials (20, 39). The features and results of important recent trials are highlighted below. Kanowski *et al.* (40) treated 216 (156 with complete data) presenile and senile primary degenerative dementia of the Alzheimer type (DAT) or multi-infarct dementia (MID) patients with 240 mg EGb 761 or placebo for 24 weeks. On the basis of measures of psychopathology, syndrome-related cognitive performance (i.e., attention and memory functions) and behavior (i.e., independence and coping skills), they concluded that GBE was of clinical efficacy in outpatients with DAT or MID. Side effects were minor. Differential effects attributed to treatment were also noted for EEG-mapping characteristics. Mauer *et al.*, (41) examined 20 outpatients with DAT treated for 3 months with 240 mg/day EGb 761 or placebo. Although the active-treatment group had poorer baseline performance measures than the placebo group, the GBE-treated group experienced an improvement in scores whereas the placebo group deteriorated during this time. In addition to these clinical performance measures, trends were seen for topographic normalization in EEG measures believed to be indicative of local effects in the CNS.

Winther *et al.* (42) examined the effects of GBE on age-related cognitive dysfunction (MMSE range 22–28) in 60 elderly subjects (58–92 years of age). They utilized a GBE product referred to as "GB-8" at a low dose (40 mg TID) or a high dose (80 mg TID) versus a placebo condition with neuropsychological testing at 1 and 3 months. In addition, they employed subjective measures of memory completed by the subjects themselves as well as a close relative. Improvements were observed in attention, concentration and short-term verbal memory in the low dose group only with changes apparent at the 1 month follow-up with statistical significance achieved at the 3 month evaluation. In addition, diastolic blood pressure was significantly reduced in the low dose group only. The authors hypothesized that GBE displays a bell-shaped dose-response curve with 120 mg/day being an optimal dosage. The 240 mg/day resulted in side effects in some subjects such as sleep disturbances, dizziness, and dyspepsia. Since the authors did not use a standardized product or provide content specifications on the "GB-8" product, direct dose comparisons with other GBE studies are difficult, however, this study provides the only direct human evidence of a bell-shaped rather than a sigmoidal dose-response curve for GBE effects.

In the largest, longest controlled trial of GBE, LeBars *et al.* (43) studied 309 (202 with complete data) patients with mild to moderately severe forms of DAT or MID. Patients were treated with EGb 761 120 mg/day or placebo. Evaluations were performed at 12, 26 and 52 weeks of therapy. The authors concluded that GBE was safe and capable of stabilizing cognitive performance and social functioning in patients with dementia, even observing improvement in some patients. Although the changes were classified as "modest," they were objectively quantifiable and noticeable by caregivers. Recent reviews of the literature on the treatment of dementia with GBE led Wettstein (44) to conclude that GBE had comparable efficacy to the cholinesterase inhibitors (tacrine, donepezil, rivastigmine, metrifonate).

However, Curtis-Prior *et al.* (45) highlighted the need for objective measures in GBE efficacy assessments in dementia research. In a further, retrospective

evaluation of their data, LeBars *et al.* (46) found that the relative change in measures were highly dependent on the initial severity at baseline. Improvement was seen in patients with "very mild" to "mild" cognitive impairments, whereas, those patients initially characterized as having "severe" dementia only experienced a stabilization or slowing of deterioration compared to placebo. In contrast to the findings of LeBars, van Dongen *et al.* (47) reported no effect of EGb 761 treatment in a heterogeneous population of older persons ($N = 214$) with mild to moderate dementia, either DAT or vascular dementia, or age-associated memory impairment. Doses of 160 and 240 mg/day were compared to placebo for 12 and 24 week periods. No benefits were detected for any subgroup, dose or period of treatment. Treatment-specific rates of adverse events were 56, 52, and 44% and 46, 40, and 65% for the 240 mg, 160 mg, and placebo doses for the first and second phases of the trial, respectively. However, only one event was considered to be associated with the study treatment and that event was in a placebo user.

MECHANISM OF ACTION: HUMAN WORK

No human studies are available directly documenting an increase in cerebral glucose metabolic rate or improvements in CNS vascular tone with GBE therapy. Two preliminary studies exist regarding changes in cerebral blood flow with GBE therapy. The first, published in 1973, found a variety of CBF responses (i.e., no change, decrease, increase) in selected individuals with cerebrovascular insufficiency using the xenon-133 technique (48). As this paper is only available in Italian, specific details beyond the figures are difficult to distinguish. The second investigation, an abstract, employed MR perfusion imaging using the GdDTPA bolus method for the semiquantitative determination of relative cerebral blood volume (CBV) and CBF in 10 subjects (61 ± 10 years) before and after GBE (120 mg/day $\times$ 4 weeks) (49). No trends were observed for any perfusion parameter to be either increased or decreased, although there was significantly more scatter in the GBE data than in the baseline data. Neither study employed a methodology capable of absolute quantitation of CBF such as [^{15}O]water PET imaging.

Cerebral Blood Flow and Metabolism

A GBE-induced increase in CBF has been based on two assumptions: 1) if cognitive performance improves and brain electrical activity increases, cerebral blood flow (and correspondingly, cerebral metabolic rate) must have increased; and 2) if GBE induces peripheral blood flow increases, then, GBE must also induce cerebral blood flow increases. The first assumption may or may not be true, depending on the cognitive function being observed. More efficient cerebral processing may actually lead to decreases in cerebral blood flow and cerebral metabolic rate rather than increases. (50) The second assumption is based primarily on work in patients with peripheral arterial occlusive disease (pAOD). The report by Schweizer and Hautmann (51) evaluating the efficacy of EGb 761 for pAOD Fontaine's Stage IIb is consistent with other reports showing significantly improved pain-free walking distances without significant changes in Doppler pressure measurements. The improvement in circulation is hypothesized to be due to actions modifying microcirculatory parameters such as numbers of perfused capillaries, erythrocyte velocity and deformability, and prevention of oxidative stress. On the other hand, Doppler imaging has documented increases in ocular blood flow in healthy volunteers treated with GBE 40 mg TID $\times$ 2 days (52). End diastolic velocity in the ophthalmic artery increased 23% over baseline with GBE versus 3% over baseline with placebo.

MECHANISM OF ACTION: ANIMAL/CULTURE WORK

Cerebral Blood Flow and Metabolism

Animal work has demonstrated an increase in CBF from 28 to 121% in 39 anatomically-defined rat brain structures (53) with GBE (130 mg/kg i.v.) compared to controls using autoradiographic methods. In this same study, no systematic effect was seen on regional glucose metabolic rate (−18 to 12.5% differences). However, GBE increased the blood glucose level in a dose-dependent fashion. Using an isolated rat brain preparation perfused at a constant rate and with a constant glucose concentration, the cortical glucose concentration was significantly lower in the GBE-treated brains without changes in the other substrates, including glucose-6-P. The authors concluded that GBE may inhibit glucose uptake, which when coupled with the increase in perfusion may contribute to its neuroprotective effects in cases of ischemia or hypoxia. Zhang *et al.* (54) also demonstrated increased CBF in rats treated with 100 mg/kg orally versus those treated with the vehicle alone.

The difference was observable after the first day of treatment but was statistically significant from the fourth day through preocclusion determinations. After occlusion of the MCA, there were no significant differences between the two groups at any of the measurement times during ischemia or reperfusion although the GBE-treated animals had higher CBF values. However, the GBE-treated animals had significantly smaller areas of infarction attributable to a reduction in apoptotic cell death potentially partially influenced by increases in CBF. Beneficial effects of EGb 761 (100 mg/kg/day orally) on impaired glucose metabolism in rat brains damaged by normobaric hypoxia and carotid clamping (55) and streptozotocin (56) have been demonstrated.

Duverger *et al.* (57) examined cerebral glucose utilization in 49 rat brain structures using autoradiography. Rats were pretreated with oral EGb 761 for 15 days at 50 and 150 mg/kg/day. Slight to moderate decreases in glucose utilization occurred with changes not exceeding 18.4 and 11.7% at the 50 and 150 mg/kg dosing levels, respectively. Statistically significant decreases were observed in the frontoparietal somatosensory cortex, nucleus accumbens, cerebellar cortex, and pons with the 50 mg/kg dose only. These findings are consistent with the utility of GBE in somatosensory processing and vestibular syndromes. This study also implies the potential for a bell-shaped dose-response curve as hypothesized by Winther (42) but the dosages employed are not comparable (see comments below).

Cerebrovascular Tone

Changes in CNS vascular tone as a possible effect of GBE therapy are also not based on direct measures of cerebrovascular responsivity, but are assertional explanations of CNS mechanisms and extrapolations from measured peripheral effects. The product, Ginkor Fort, a combination of GBE, troxerutine and heptaminal, demonstrated a beneficial action on the venous wall as measured by the circulating endothelial cell count in patients with chronic venous insufficiency (58). Furthermore, the effects on vascular tone may be an indirect effect of the antioxidant action of GBE. Experimental vasospasm and vasculopathy were evaluated in a double hemorrhage dog model of chronic cerebral vasospasm by Kurtsoy *et al.* (59). The EGb-761-treated (100 mg/kg i.v.) animals had significantly smaller decreases in vessel diameters and none of the histopathological signs of proliferative vasculopathy when compared to the control animals. The authors concluded that the protective effect of GBE against subarachnoid hemorrage-induced vasospasms and vasculopathy were the result of the antioxidant effects. GBE has been documented to inhibit NO production in a human endothelial cell line (60). Unbalanced NO production has been observed in various pathological processes including atherosclerosis and endothelial dysfunction. In addition, EGb 761 has been shown to inhibit NO production and also act as a NO scavenger under the condition of ischemia/reperfusion in working rat hearts. This reduction in NO improves the recovery of postischemic heart function (61).

Antioxidant/Free-Radical Scavenger

The damaging effects of oxidative insults and free-radical formation on tissues is a growing area of investigation. A number of intriguing recent studies highlight the potential of GBE and its constituents as antioxidants and free radical scavengers. The prevention of the cerebral ischemia-induced Na, K-ATPase injury and lipoperoxidation by the free-radical scavenger properties of EGb 761 have been documented (62) as well as protection against the apoptosis of cerebellar cells (5, 63) and activation of nuclear factor kappa B in vascular endothelial cells induced by hydroxyl radicals (64). A protective effect on myocardial and skeletal muscle tissues (microvessels and interstitium) in diabetes has been demonstrated (65, 66). EGb 761 especially appears to protect mitochondrial ATP against anoxia/reoxygenation injury (67, 68). The possible role of mitochondrial dysfunction in Alzheimer's disease was recently reviewed (69). Reactive oxygen species-induced apoptosis was significantly reduced in EGb 761-treated mice, especially in the older animals (70). GBE and bilobalide, a component with possible anticonvulsant properties, appears to especially protect hippocampal tissues possibly through GABAergic, NO, or membrane-based mechanisms (7, 8, 71, 72–74).

In neuronal cell culture, pretreatment with GBE did not protect against β amyloid-induced apoptosis and cell death, although preventing the β amyloid reactive oxygen species generation (75, 76). Whereas, cotreatment with EGb 761 protected, in a dose-dependent manner, the neuronal cells from β amyloid-induced cell death (77) possibly via blocking the formation of β amyloid-derived diffusible neurotoxic ligands (ADDLs) or effects on cellular

glucose metabolism (76). Using a neuroblastoma cell line expressing an Alzheimer's disease mutation, Luo *et al.* (78) reported that EGb 761 inhibited for formation of β amyloid fibrils and significantly attenuated mitochondrion-initiated apoptosis. In addition, the activity of caspase 3, an apoptosis cell-signalling enzyme, was decreased by EGb 761. The effects of β-amyloid play a leading role in the hypothesis for the cause of Alzheimer's disease.

Neuroprotective Effects

Animal studies have demonstrated enhanced cognitive performance as measured by memory tasks in mice (79) and young and old rats (80). Acute doses of EGb 761 (10 mg/kg i.p.) have also improved stimulus control in rats, an effect that was mediated by activity at the 5-HT_{1A} receptor (81). Further neuroreceptor work indicates that treatment with EGb 761 (20–100 mg/kg/day i.p. $\times$ 7 days) but not ginkgolides A or B (10 mg/kg/day i.p.) protected mice against the neurotoxin, MPTP, implicated in the pathogenesis of Parkinson's Disease (82). EGb 761 protected against the amphetamine behavioral sensitization, possibly via effects on glucocorticoid levels (71). The MAO-inhibitory effects, especially MAO-B, were hypothesized to be the mechanism of this neuroprotective effect. Further work by Pardon *et al.* (83) also found evidence of a neuroprotective effect of EGb 761 (50 mg/kg) against stress-induced increases in MAO activity in mice. Sloley *et al.* (11) attributed MAO-inhibiting activity to the kaempferol component of the extract. Although Porsolt *et al.* (84) did not find any evidence of MAO inhibition activity in mice.

Mechanism of Action: Human Imaging Work

Intrigued by the possible inhibitory effects of GBE on MAO A and B, Fowler *et al.* (85), investigated their levels in the living human brain with PET imaging and the radiolabelled MAO suicide inactivators [^{11}C]clorgyline and [^{11}C]L-deprenyl-D2, respectively. One month of treatment at 120 mg/day revealed no change in either MAO A or B levels. The authors hypothesized that differences observed between in vitro and in vivo work in animal species and humans may be due to the need for the GBE to cross the blood brain barrier. In vitro results must be viewed as only preliminary before confirmation by in vivo results in humans. The authors also noted that there was evidence of increased clotting times (after removal of the arterial catheter) and no evidence in the K_1 model parameter (transfer constant related to brain blood flow) of a change in tracer delivery to the brain, one component of which is cerebral blood flow. However, they stated that "a more direct measurement of brain blood flow would be required in order to definitely rule out changes in blood flow. In addition, the reactivity of the cerebral vasculature may differ in normal subjects compared to elderly subjects and those with dementia and this also needs to be considered."

SCIENTIFIC RESEARCH NEEDS

The work of Fowler *et al.* (85) highlights the inherent short-comings of extrapolations to human pharmacology of in vitro and in vivo animal studies of CNS-active drugs. Especially for herbal agents that are a combination of a number of potentially active substances, as GBE is, the observed response will be a complex function of the pharmacology, pharmacokinetics, and pharmacodynamics of the individual components as well as the synergy of the combinations. Furthermore, dosages utilized in studies must reflect reasonable human dosages, not the extraordinarily high dosages used in animal and in vitro work. This is especially true for agents that exhibit a bell-shaped rather than a sigmoidal dose-response relationship. For example, the common dosage of EGb 761 used in a large amount of the animal work is 100 mg/kg/day orally, i.v. or i.p. This dose would translate into 117 standard 60 mg tablets for a 70-kg individual per day. Animal research with humanly-realistic dosages needs to be carried out in order to both rule in as well as rule out potential pharmacological effects of GBE.

CONCLUSIONS AND FUTURE DIRECTIONS

Cognitive deterioration will perhaps constitute the single biggest public health problem of the next several decades as the demographics of our nation shift to later life. While GBE appears to be safe, the question of effect, especially in the healthy population, remains scientifically unresolved. See Table 1 for a synopsis. Whether GBE alters cerebral blood flow, cerebral metabolic rate and/or cerebral vascular reserve thereby improving age-related cognitive declines or if there is a cognitive benefit from increasing cerebral blood flow or cerebral metabolic rate in

Table 1. Synopsis of *Ginkgo biloba* Effects on the Central Nervous System

Potential indication	Evidence
Based on human studies	
Augmentation of cognitive function in healthy subjects	Mixed results. In studies with positive findings, effects potentially more pronounced for "working memory" and "speed of processing" and more apparent in older subjects.
Augmentation of cognitive function in impaired or demented subjects	Mixed results. In studies with positive findings, GBE was generally safe and stabilized cognitive performance and social function. Improvement was more likely to occur in patients with mild rather than severe cognitive impairments.
Increase in cerebral blood flow	Variable results. Little direct human evidence of central effects, mechanism primarily based on peripheral measures.
Improvements in CNS vascular tone	No direct human evidence.
Increase in glucose metabolism	No direct human evidence.
Based on animal studies	
Increase in cerebral blood flow	Evidence of increased cerebral blood flow at high i.v. doses (≥100 mg/kg).
Improvements in CNS vascular tone	May be an indirect effect of antioxidant actions.
Increase in glucose metabolism	Variable effects on regional glucose metabolic rates, possibly dose-related. Trends toward reductions in glucose metabolism which may be neuroprotective in cases of ischemia or hypoxia.

healthy older adults in the absence of dementia remains unclear (86).

At the present time, there are no definitive treatments for dementias or age-related cognitive decline. The significance of these disorders mandates that every therapeutic option be investigated with rigorous scientific methodology. The pharmacological potential of GBE will only be completely understood through coordinated in vitro/in vivo, animal investigations coupled with human placebo-controlled, double-blinded research designed to objectively measure relevant functional parameters.

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