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Studies on molecular mechanisms of *Ginkgo biloba* extract

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Abstract In the past decade, interest by the general public in the use of herbal dietary supplements has risen exponentially. As throughout history, individuals are now turning to the use of “natural” therapies for the prevention, treatment and cure of almost every ailment and aging malady imaginable... often without substantial proof of safety or efficacy. One of the most popular herbal supplements is *Ginkgo biloba* extract, taken for its perceived “memory enhancing” properties. Given the inordinate popularity, growing use, and substantial number of pharmaceutical products containing *G. biloba*, coupled with demands for product safety and “hard evidence,” science has followed this trend closely with an ever-expanding body of pharmacological and clinical data on such preparations. Claims that standardized *G. biloba* extract (EGb 761) can modulate the cellular environment of an organism under both physiological and stress conditions may be attributed to its multivalent or totipotent properties, and can now be substantiated by the availability of modern molecular techniques. As opposed to pharmacologically manufactured or synthetic drugs, which provide a single target for a single receptor as the mechanism of action, EGb 761 is able to up- or down-regulate signaling pathways, gene transcription, cellular metabolism, etc., and thus assist in the regulation of the general physiological status of the cell and/or organism in response to stressors posed by both intracellular and extracellular conditions. Presumably, this is one of the biggest advantages of using natural products for the prevention and treatment of infirmity, as well as the maintenance of health in an organism.

The new age of nutraceuticals embraces a centuries-old herbal extract: EGb 761

According to consumer surveys, a steadily growing trend in the United States is the widespread use and growing acceptance of unconventional medical therapies and/or alternative medicine. At least 50–75% of the United States population has tried alternative therapies according to one survey (Neldner 2000). In a 1998 study published in the *Journal of the American Medical Association*, 42% of Americans were using alternative medicine, representing an 8% increase from 34% in 1990.

Currently, the use of complementary/alternative medicine (CAM), inclusive of vitamins, dietary supplements, herbal therapies and alternative medicine professional services, represents an industry ranking second in expenses being paid out-of-pocket, with a 45.2% increase in expenditures between 1990 and 1997 estimated at US \$21.2 billion in 1997 (Eisenberg et al. 1998). Dietary supplements are used by more than one-half of the adult population in the United States (Halsted 2003). Around the globe, individuals in a wide array of age groups, lifestyles and states of health or infirmity are using CAM with expectations of beneficial health improvements, boosted immunity, enhanced sports performance and overall perception of well-being. Far from being “new age,” these expectations may well be rooted in a long history of “natural therapies” passed down from person to person as well as in documented use over hundreds of years. Indeed, historical analysis of ancient cultures is rich in the use of phytomedicines as curative agents of disease, and modulators of overall health and vitality.

Traditional Chinese medicine has a long history of treating common ailments with natural therapies in order to eradicate illness and restore balance within the body. For over 5,000 years, the fruits and seeds of the *Ginkgo biloba* tree (Fig. 1) have been used in traditional Chinese medicine, with indications for the treatment of asthma, cough, and enuresis (Zimmermann et al. 2002). Much later, use of *Ginkgo* leaves came into practice for treating skin infections. The first publication concerning the

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internal use of the leaves of the *Ginkgo* tree for medicinal purposes dates back to 1505 A.D. in a text by Liu Wen-Tai, Ben Cao Pin Hue Jing Yaor (see DeFeudis and Drieu 2000). Since the early 1990s, the standardized extract of *G. biloba* leaves, EGb761, is becoming one of the most popularly used supplements for memory enhancement in the United States.

An overview of the current state of *G. biloba* extract EGb 761

The standardized extract: Why should there be one?
What's it made of?

Depending on the country of origin, time of harvest, etc., the percentages of individual constituents found in *G. biloba* can vary considerably; therefore it is highly desirable to have a standardized preparation with a known constituent makeup for the purposes of clinical trials, drug regulation, human consumption and for purposes of research and reproducibility. French and German companies have approved a standardized form of *G. biloba* leaf extract (EGb 761, developed by Beaufour-Ipsen Pharma (Paris, France) and Dr. Willmar Schwabe Pharmaceuticals (Karlsruhe, Germany) that contains 24% flavonoid glycosides (Fig. 2A), 6% terpene lactones (Fig. 2B), and less than 5 ppm ginkgolic acid, the



Fig. 1 Photograph (courtesy of Weiss and Fintelmann 2000) of *Ginkgo biloba* leaves (*Ginkgo biloba folium*) and fruit. As the only surviving species member of the 200 million-year-old order Ginkgoales (class Gymnospermae), which coexisted with the dinosaurs during the Jurassic period, the *G. biloba* tree is considered a living fossil (DeFeudis and Drieu 2000). *Ginkgo* trees can grow up to 100 feet (30.5 m) tall with a 50 feet (15.25 m) circumference, and have been known to live up to 4,000 years, possibly due to their genetic tenacity to be disease resistant and high tolerance for withstanding polluted environments (Zimmermann et al. 2002)

component that has allergenic properties (Jacobs and Browner 2000). One documented method of obtaining EGb 761 from *Ginkgo* leaves is as follows: The leaves of *G. biloba* trees are collected while still green from July to September, dried, then analyzed morphologically, microbiologically and chemically for an evaluation of the presence of heavy metals, pollutants and constituents (Bilia 2002; Zimmermann et al. 2002). Next, a refinement process includes the extraction and concentration of the active constituents from the crude dried leaves formulation with an acetone-water mixture. Lastly, the flavonoid fraction is analyzed through HPLC/UV techniques, while the terpenes are identified and quantitated by HPLC coupled with a retention index detector (Bilia 2002; Zimmermann et al. 2002). As a dietary supplement in the United States and available by prescription in European countries, an extract made from the leaves of the *G. biloba* tree topped the list of the seven best-selling herbal medicinal products in the United States with 1998 retail sales of US \$151 million [St. John's wort (\$140 M), ginseng (\$96 M), garlic (\$84 M), echinacea (\$70 M), saw palmetto (\$32 M), and kava (\$17 M)] (Ernst 2002).

The components of EGb 761, and what they do

The two major fractions of the extract, the terpenes and the flavonoids, having separate properties, are responsible for giving this extract its unique polyvalent pharmacological action. The terpene lactones are represented by the ginkgolides A, B, C, J and M and bilobalide (Fig. 2B). The ginkgolides are platelet-activating factor (PAF) antagonists, able to reduce platelet activation and aggregation, and therefore having the potential to improve blood circulation. Bilobalide, a sesquiterpene trilactone constituent of *G. biloba* leaf extracts, can reduce cerebral edema produced by triethyltin, decrease cortical infarct volume in certain stroke models, and reduce damage from cerebral ischemia (DeFeudis 2002a). The increase in blood circulation allows increased delivery of oxygen and glucose to the brain following an ischemic event.

The antioxidant effect of the flavonoid fraction may be achieved by either direct attenuation of reactive oxygen species (ROS) (Smith and Luo 2003), chelating pro-oxidant transitional metal ions (Gohil and Packer 2002), expression of antioxidant proteins such as superoxide dismutase (SOD), and increase in antioxidant metabolites such as glutathione (Gohil and Packer 2002; Oken et al. 1998). The flavonoid fraction of the extract appears to be more effective against hydroxyl radicals than the terpene fraction (Bastianetto et al. 2000; Zimmermann et al. 2002). The chemical structure of the flavonoids in general is comprised of an aromatic ring and a double bond (Fig. 1A). Therefore, the flavonoids react preferentially with, and directly scavenge, the hydroxyl radicals (Zimmermann et al. 2002). Additionally, the phenolic hydroxyl groups on the flavonoids may be able to chelate pro-oxidant transition heavy metal ions (Gohil and Packer 2002) (e.g., Fe^{2+}), consequently inhibiting the formation

A Flavonoid glycosides obtained from *Ginkgo biloba*.

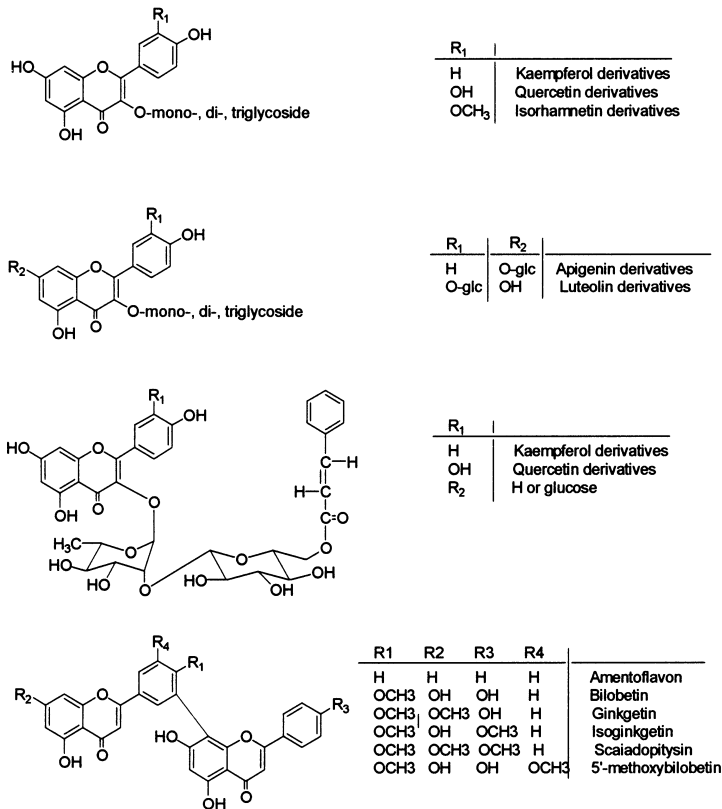


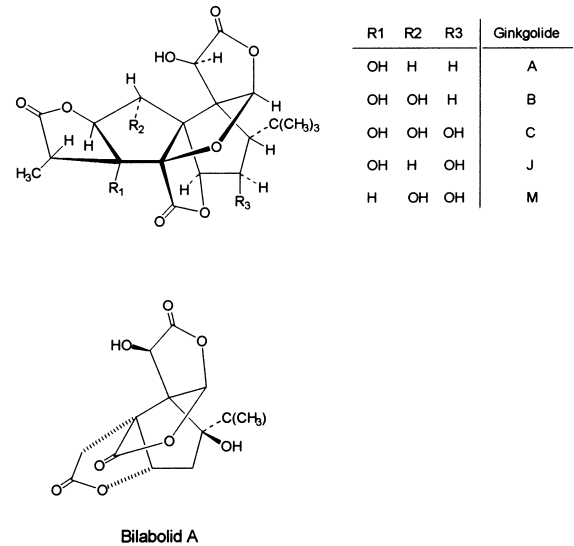
Fig. 2 A Chemical structure of flavonoid constituents. Essentially flavonol-*O*-glycosides, with a glycosidic linkage normally located in position 3 or 7 of a phenolic aglycon (quercetin, kaempferol or isorhamnetin) and the carbohydrate moiety usually being *D*-glucose,

of new hydroxyl radicals (Ni et al. 1996; Zimmermann et al. 2002).

Some major biochemical/pharmacological activities of EGb 761

- 1 Free radical scavenger activity, thereby decreasing tissue levels of ROS (DeFeudis and Drieu 2000; Lien et al. 1999; Smith and Luo 2003) and inhibition of membrane lipid peroxidation (DeFeudis and Drieu 2000)
- 2 Anti-PAF activity, contributing to improvements in cerebral insufficiency (Smith et al. 1996)
- 3 Stimulation of endothelium-derived relaxing factor (EDRF) resulting in improved circulation in arteries, veins, and capillaries (DeFeudis 1998)
- 4 Decrease in the expression of peripheral benzodiazepine receptor (PBR) of the cerebral cortex (DeFeudis and Drieu 2000)
- 5 Bilobalide component increases the respiratory control ratio of mitochondria by protecting against the uncoupling of oxidative phosphorylation, thereby

B Terpenylactones (ginkgolides and bilobalide) of *Ginkgo biloba*.



L-rhamnose or glucorhamnose. **B** Chemical structure of terpene trilactone constituents of EGb 761, occurring exclusively in the *Ginkgo* tree (DeFeudis and Drieu 2000)

increasing ATP levels (DeFeudis 2002a)

- 6 Enhances neuronal plasticity (DeFeudis and Drieu 2000; Gohil and Packer 2002)
- 7 Anti-inflammatory effects and protective actions against brain damage, probably through its terpenoids and ginkgolides (Oberpichler et al. 1990)
- 8 Attenuates apoptosis in neuronal cultures (Bastianetto et al. 2000; Luo et al. 2002; Smith et al. 2002)
- 9 Inhibition of A β aggregation in neuroblastoma cells (Luo et al. 2002)
- 10 Increases stress resistance and extends organism's life span in *Caenorabditis elegans* (Wu et al. 2002) and mice (Ward et al. 2002; Winter 1998)

Some major clinical applications

- 1 Improvement of peripheral arterial insufficiency (DeFeudis and Drieu 2000)
- 2 Treatment of cerebral disorders due to aging, including cognitive decline, short-term memory and neurosensory impairments not associated with dementia (DeFeudis 2002b)

- 3 Treatment of tinnitus, acute cochlear deafness, vertigo, and disturbances in equilibrium (DeFeudis and Drieu 2000; Meyer 1986)
- 4 Counteracts the cognitive deficits that follow stress or traumatic brain injury (DeFeudis and Drieu 2000)

Neuroprotection studies

Many basic science and clinical studies have been performed, both in vitro and in vivo to support the use of, and identify therapeutic indications for, *G. biloba* extract EGb 761. The neuroprotective effects of EGb 761 have been substantiated by many research groups (Luo 2001). EGb 761 has several major pharmacological and therapeutic actions: it enhances cognition, improves blood rheology and tissue metabolism, and opposes the detrimental effects of ischemia (DeFeudis and Drieu 2000); with additionally noted improvements in neurophysiological and psychometric measurements in patients with cerebro-organic syndrome (Lien et al. 1999). Animal studies have revealed that EGb 761 is able to facilitate acquisition and retention of memory (Cohen-Salmon et al. 1997; Winter 1998), with one of the major protective actions taking place in the hippocampus (Barkats et al. 1995), which is related to the acquisition of new memories (Squire 1992).

In 2000, Francis DeFeudis proposed several mechanisms of action that are useful in explaining how EGb 761 benefits patients with Alzheimer's disease (AD) and other age-related, neurodegenerative disorders. In animals, EGb 761 possesses antioxidant and free radical-scavenging activities; it reverses age-related losses in brain alpha 1-adrenergic, 5-HT_{1A} and muscarinic receptors; protects

against ischaemic neuronal death; preserves the function of the hippocampal mossy fiber system; increases hippocampal high-affinity choline uptake; inhibits the down-regulation of hippocampal glucocorticoid receptors; enhances neuronal plasticity; and counteracts the cognitive deficits that follow stress or traumatic brain injury (DeFeudis and Drieu 2000).

Anti-apoptotic properties of EGb 761

Apoptosis has been related to neurodegenerative diseases (Yuan and Yankner 2000). The induction of apoptosis in a cellular system by subjection to multiple cytotoxic factors is effected through several molecular pathways. We have observed in a PC12 neuronal system that the neuroprotective properties of EGb 761 are multifactorial, and act synergistically upon multiple cellular pathways (Smith et al. 2002). These observations included: the ability of EGb 761 to protect the pivotal integrity of the mitochondrial membrane; attenuate the release of cytochrome *c* from the mitochondria, a critical step in the formation of the apoptosome leading to the initiation of the apoptotic caspase cascade; up-regulate the transcription of anti-apoptotic Bcl-2-like protein; down-regulate the transcription of pro-apoptotic caspase-12, an endoplasmic reticulum specific stress mediated protein; inhibit the cleavage and activation of caspase-3, a key effector protease in the execution of apoptosis; and inhibit nuclear DNA fragmentation, the hallmark of apoptosis (Fig. 3) (Smith et al. 2002). Thus, the polyvalent activity of EGb 761 provides neuroprotection to a cellular system at least partially

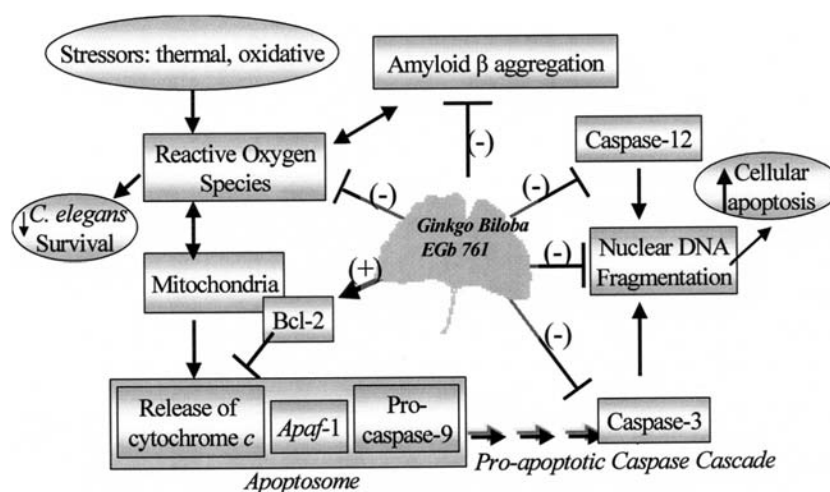


Fig. 3 Proposed neuroprotective action of EGb 761. We have observed the anti-apoptotic properties of EGb 761 in vitro via the maintenance of the mitochondrial membrane potential, up-regulation of antiapoptotic Bcl-2 protein, and subsequent inhibition of both the formation of the apoptosome and activation of the pro-apoptotic caspase cascade leading to programmed cell death, which includes nuclear DNA fragmentation. Additionally, expression of caspase 12, an endoplasmic reticulum-stress specific protease, is down-regulated by EGb 761 treatment (Smith et al. 2002). We have observed the

anti-oxidative properties of EGb 761 in transgenic cells and *Caenorhabditis elegans* models through direct attenuation of hydroxyl radicals-related reactive oxygen species (ROS) levels (Smith and Luo 2003), and imparting increased stress resistance and longevity in model organisms (Wu et al. 2002). We have observed the anti-A β aggregation properties of EGb 761 through decreased A β aggregation in the conditioned medium of an A β -secreting cell line, and also by directly inhibiting the aggregation of A β fibrils in a cell-free solution in vitro (Luo et al. 2002)

through bi-directional mediation of the existing apoptotic machinery.

Effects of EGb 761 on amyloid β aggregation

Early onset, autosomal dominant forms of AD appear to be caused or exacerbated by deposition in the brain of amyloid β ($A\beta$), a proteolytic fragment of 40–42 amino acid residues derived from amyloid precursor protein (APP) (Gandy et al. 1994). Mutations in APP and presenilins (PS1, PS2) associated with familial AD, are known to increase the production of the total $A\beta$ or the more amyloidogenic form $A\beta_{42}$ (Price and Sisodia 1998). Using a neuroblastoma cell line stably expressing an AD-associated mutation, we reported that EGb 761 is able to inhibit the formation of $A\beta$ aggregates (Fig. 3) both in the conditioned medium of this $A\beta$ -secreting cell line and directly in solution (Luo et al. 2002). Hence, these results suggest that direct inhibition of $A\beta$ aggregation may underlie an additional neuroprotective effect of EGb 761.

Effects of EGb 761 on free radical levels and oxidative stress

With an ever-increasing emphasis on maintaining the health and vitality of an aging population, the effect of free radicals on aging and the development of illness has become a major research area. According to the free radical theory of aging, administration of antioxidants may slow the aging process as well as age-associated impairment at the cellular and tissue levels; therefore the antioxidant actions of EGb 761 make it an excellent candidate as an anti-aging “drug” (Sastre et al. 2002). In the nematode *C. elegans* fed with EGb 761, we demonstrated increased resistance of wild-type worms to acute oxidative and thermal stress of 33% and 25%, respectively (Wu et al. 2002). Thus, our results showed that oxidative stress, a major determinant of life span, as well as other types of stress and physiological impairment associated with aging, may be successfully counteracted by EGb 761 (Fig. 3).

The production of ROS through normal aerobic metabolism has been postulated to be a major contributor to the aging process, as well as contributing to neurodegenerative pathologies, such as those in AD. Many studies elucidating the effects of EGb 761 on the molecular level have been followed by animal studies for verification of the hypothesis. The assumption that the beneficial action of EGb 761 is due to its free-radical scavenging effects has been shown in numerous in vitro studies, and increasing numbers of in vivo studies. For example, using genetically engineered models expressing AD mutations, we recently reported that ROS production was significantly higher both in an in vitro cellular AD model, and also in an in vivo *C. elegans* AD model; and that pre-treatment with EGb 761 resulted in significantly lower ROS levels in both models (Fig. 3) (Smith and Luo 2003). The radical

scavenging effects of EGb 761 have been directly verified by the reduction of lipid peroxidation in mouse brain tissue with experimental cerebral ischemia (Pierre et al. 1999) and even in humans during open-heart surgery (Pietri et al. 1997).

Transcriptional effects of EGb 761 using gene microarray technology

Modern molecular biology is increasingly able to provide researchers with the tools necessary to decode the diverse effects of complex natural substances on biological systems. Changes in gene expression and protein interaction profiles can be studied using genomics and proteomics microarray methods (Blohm and Guiseppi-Elie 2001), in which the activity of several thousand genes can be studied at once. Evidence from recent studies indicate that the therapeutic effects of EGb 761 likely involve the modification of gene expression through the complex interaction of extract components, and in some cases through the actions of its pharmacologically active chemical constituents (DeFeudis 2002b). These gene array assays, which provide observations of changes in gene expression, yield molecular evidence for the action of EGb 761 in the brain, and show differential effects of the extract in separate brain regions (Watanabe et al. 2001). The heterogeneity in the responsiveness of different neuroanatomical brain regions raises an issue for future research regarding the targeting of specific brain regions by herbal medicine and/or dietary supplementation in order to achieve a desired molecular, physiological, and behavioral outcome (Gohil and Packer 2002).

Reports from microarray assays support the neuroprotective effects of EGb 761. Together, these findings indicate that EGb 761 modulates the transcription of selected genes responsible for the initiation of an adaptive response (Gohil et al. 2000), thereby increasing the antioxidant status of the cells, and improving an organisms cellular tolerance to oxidative stress (DeFeudis 2002b). Further, EGb 761 inhibits DNA damage through modulation of proteins involved in DNA synthesis, repair and cell cycle function (DeFeudis 2002b).

In our recent study, the anti-apoptotic mechanisms hypothesized to play a role in the neuroprotective properties of EGb 761 were supported by DNA microarray analysis (Table 1). In this study, microarray was used to quantitate changes in gene expression in neuronal growth factor (NGF) differentiated PC12 cells treated with EGb 761. Our study demonstrated that EGb 761 increased the expression of an anti-apoptotic Bcl-2 protein, and altered the transcription of multiple apoptotic-related genes in PC12 cells, including the down-regulation of a pro-apoptotic protein, caspase-12 (Fig. 3) (Smith et al. 2002). Watanabe et al. (2001) measured the effect of EGb 761 on gene transcription in mice using microarray analysis. The hippocampal and cerebral cortex tissue from mice fed a diet supplemented with EGb 761 for 4 weeks showed ten genes up-regulated by 3-fold or more. The neuromodula-

Table 1 Some transcriptional changes resulting from administration of EGb 761, as identified by DNA microarray technology. Selected gene products with at least a 2-fold change in transcription

Gene product	Effect	Reference
Mitochondrial SOD	Increase of transcription; antioxidant enzyme	Gohil and Packer 2002; Gohil et al. 2000
Heme oxygenase-1	Increase of transcription; antioxidant	Chen et al. 2001; Gohil and Packer 2002; Gohil et al. 2000; Zhuang et al. 2002
Transthyretin	Stimulated expression in vivo in the hippocampus; sequestration of Amyloid β ; hormone transport	Gohil and Packer 2002; Watanabe et al. 2001
P65 and Hsp70	Increase due to reduction of glutathione reductase and glutathione S-transferase; chaperone proteins	Soulie et al. 2002
NADH dehydrogenase subunit 1	Stimulated expression in PC12 cells—decreases state-4 respiration, whose increase is thought to be responsible for oxidative damage	Tendi et al. 2002
Cytochrome-c oxidase	Up-regulated—providing protection in cerebral ischemia in gerbils	Chandrasekaran et al. 2001; Spinnewyn et al. 1995
Bcl-2 interacting protein	Increased of transcription; anti-apoptotic effect; inhibits the activation of the pro-apoptotic caspase cascade downstream	Gohil and Packer 2002; Smith et al. 2002
Caspase 12	Decreased transcription, whose increase is thought to be directly related to endoplasmic reticulum stress	Smith et al. 2002
18 kDa Peripheral benzodiazepine	Decreased transcription; antistress effect; decreased corticosteroids; neuroprotection	Amri et al. 1996, 2002

due to EGb 761 treatment are shown based on microarray analysis, along with general effects of these changes in cellular and organism models. *SOD* Superoxide dismutase

tory effects of EGb 761 were shown in these mice by the significant upregulation of transthyretin, AMPA-2 channel, neuronal tyrosine/threonine phosphatase 1, and microtubule-associated τ , all of which may have neuroprotective roles (Watanabe et al. 2001).

Similarly, Soulie et al. (2002) defined the transcriptional effects of EGb 761 on human hNT neurons by using cDNA macroarrays, including genes implicated in antioxidant and stress responses. The group identified seven genes whose expression was strongly modified by the EGb 761 treatment. From these seven, three groups can be distinguished: genes encoding transcription factors (increase in NF-kappaB p65 subunit and zinc finger protein 91 mRNAs, and decrease in c-myc transcripts), genes involved in antioxidant defenses (increase in CuZn SOD mRNAs, and decrease in glutathione reductase and glutathione S-transferase pi mRNAs) and genes involved in stress responses (up-regulation of HSP70 transcripts) (Soulie et al. 2002).

Effects of EGb 761 on stress modification, mood and memory

In addition to the enhancement of memory found in numerous studies, researchers studying AD with various animal models have found EGb 761 to be beneficial in targeting other essential components of cognition that may be altered with age and infirmity, such as decision-making ability (Pardon et al. 2000), response of aged animals to training of new skills (Cohen-Salmon et al. 1997), and response to stress and mood changes.

In a series of tests of acquisition, performance and retention, Winter (1991) found that EGb 761 not only facilitated memory processes in mice, but that the treated animals also demonstrated improved retrieval of the

learned responses. Blavet (1992) found that treatment with EGb 761 enhanced learning parameters in aging rats tested on an eight-arm radial maze. Other studies of learning and memory have shown that treatment of mice with EGb 761 improves short-term memory in a passive avoidance paradigm and increases brain neuronal membrane fluidity, which may be a function of the anti-oxidant and anti-lipoperoxidative properties of EGb 761 (DeFeudis and Drieu 2000).

The effects of stress can either be beneficial or detrimental to an organism. Chronic stress, however, often results in decline of physical performance, emotional state, and physiological functioning. In an unexpected observation, Ward et al. (2002) found that laboratory rats treated with EGb 761 showed significantly less stress and anxiety response compared to untreated control animals when subjected to the Morris Water Maze. This result is supportive of previous “anti-stress” findings of EGb 761, which postulate that the anti-stress effects of EGb 761 act via its ginkgolide constituents to decrease the expression of the PBR of the adrenal cortex (Amri et al. 1996, 2002; DeFeudis and Drieu 2000), leading to a reduction in corticosteroid synthesis (Amri et al. 2002), and the regulation of cerebral monamine oxidase activity (Pardon et al. 2000).

In addition to the anti-stress action of EGb 761 shown in numerous animal models (Pardon et al. 2000; Rapin et al. 1994), it also appears to possess an antidepressant activity (DeFeudis 1998; Schubert and Halama 1993), thereby improving mood (Watanabe et al. 2001), which may be particularly beneficial in patients displaying behavioral and psychological disorders (BPSD) (Christen 2003). The antidepressant-like (vigilance-enhancing) and anti-stress actions of *G. biloba* extract in clinical studies have been observed at the behavioral level in both healthy

subjects and patients with depressive episodes and/or dementia (DeFeudis and Drieu 2000; DeFeudis 1998).

EGb 761 put to the test in human cognitive performance

In order for a clinical trial to be acceptable, many criteria need to be met in the design and execution of the experiment. The patient characterization must be thorough, exclusion criteria and use of a standardized EGb 761 extract must be specified; and the design of the study should be randomized, placebo-controlled, and double-blind to be scientifically relevant (Zimmermann et al. 2002).

Clinical studies have shown EGb 761 to be beneficial in the treatment of cerebrovascular insufficiency and related conditions. Results of various double-blind, placebo-controlled studies include the regression of vertigo, headache and tinnitus, improvements in short-term memory, mental alertness and dizziness, and a significantly improved score on tests such as the geriatric clinical evaluation scale (GCEs) by patients receiving EGb 761 treatment (DeFeudis and Drieu 2000).

In a study published in the *Journal of American Medical Association*, Solomon et al. (2002) found no effect on memory enhancement in elderly adults as measured by performance on 14 standard neuropsychological tests of learning, memory, attention or concentration, as well as subjective ratings by the participants and their families. From this study, Solomon suggests that *G. biloba* extract provides no measurable benefit in memory or related cognitive function in healthy adults with intact cognitive function (Solomon et al. 2002). In contrast, in a placebo-controlled, double-blind, randomized trial of *G. biloba*, Le Bars et al. (1997) reported significant improvement among patients with either multi-infarct dementia or AD on cognitive tests. The discrepancy of the two trials could be due to the short duration, use of the minimally recommended dosage of EGb 761 (120 mg/day) and the issue of blinding (Hoerr 2002). In another clinical trial of cognitively intact older adults, results from standardized, neuropsychological tests and a subjective, follow-up self-report questionnaire provided complementary evidence of the potential efficacy of a 6-week trial of *G. biloba* EGb 761 (180 mg/day) in enhancing certain neuropsychological/memory processes of cognitively intact older adults, 60 years of age and over (Mix and Crews 2002).

Trends and prospects for the future

Currently, the Ginkgo Evaluation of Memory (GEM) study in the United States, supported by NIH, and the GuidAge study in France are testing the efficacy of EGb 761 as a potentially preventative treatment for Alzheimer's disease in humans (Christen 2003).

Although gene expression profiling (molecular phenotype) of mice fed with EGb 761-supplemented diets showed significant increases in transcripts encoding brain

proteins, the behavioral phenotype of EGb 761 treatment remains to be defined in regards to health and neurological diseases (Gohil and Packer 2002). The fact that AD mutant mice, which over-express mutations responsible for AD in humans, are able to perform at the same level as wild type animals when treated chronically (>6 months) with EGb 761 (Stackman et al. 2003) is very promising. Some studies have shown behavioral changes that correlate with the molecular expression; however, the direction of future studies may be to combine functional genomics and behavioral analysis to yield an objective analysis of the in vivo effects of dietary supplementation with the *G. biloba* extract EGb 761 (Gohil and Packer 2002).

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