

Ginkgo biloba neuroprotection: Therapeutic implications in Alzheimer's disease

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An extract of Ginkgo biloba leaves, EGb761, is becoming one of the most popular dietary supplements in the United States to enhance memory. In Europe it is a commonly prescribed drug for treatment of age-related deterioration, including degenerative dementias of the Alzheimer type (AD). Substantial experimental evidence indicates that EGb761 has neuroprotective potency under conditions such as ischemia, seizures and peripheral nerve damage. However, the mechanisms of such neuroprotective effects remain unknown, partially because of the complex chemical composition of EGb761 and the resulting so-called "polyvalent" action. This review focuses on cellular and molecular approaches towards understanding the polyvalent action of EGb761 neuroprotective effect. Two potential mechanisms of action, reducing oxidative damage and stimulating cell survival machinery, are discussed. Better understanding of the neuroprotective mechanisms of EGb761 will provide impetus for possible combination therapies and for the design of rational, "mechanism-based" strategies that target age-related neurodegeneration and Alzheimer's disease.

1. Introduction

Ginkgo biloba tree, known as "a living fossil", has a life span of 4000 years, possibly due to its high tolerance to pollution and resistance to infections [17]. Extracts from the Ginkgo biloba leaves have been found in ancient and modern Chinese herbal pharmacopoeia as treatment for dysfunctions of heart and lung and as promoter of longevity [18]. Standardized extract of Ginkgo biloba leaves is presently used in Europe as one of the most commonly prescribed drugs for treatment of age-related deterioration of mental functions, as well as for treatment of vascular dementia and degenerative dementias of the Alzheimer type (AD) [39,

41]. In the United States, it is one of the most popular marketed herbal medicines, not yet under the strict regulation of the Food and Drug Administration (FDA), for enhancement of blood circulation and memory.

The standardized extract of Ginkgo biloba leaves named EGb761 contains specific percentages of ginkgo-flavone glycosides (24%) and terpenoids (6%), the later group consisting of bilobalide and the ginkgolides A, B, C, M, and J [28]. It is not certain which substances in EGb761 are responsible for the presumed health-enhancing properties. It has been suggested that the glycosides possess antioxidant activity, and the ginkgolide B, also known as BN52021, is a potent antagonist of the platelet-activating factor (PAF) receptor [60]. Accumulating evidence has suggested that many of the actions of EGb761 are so-called "polyvalent" actions, i.e., the clinical responses of EGb761 are the net effect of interactions between the various biological activities of the individual substances of EGb761 [16]. This is in agreement with the philosophy of Chinese herbal medicine, in which wholesome effects of a mixture of compounds, acting simultaneously in combination and synergy, are required to balance body's yin and yang.

2. Neuroprotective effects of EGb761

During the past decade, in vivo and in vitro experiments in mammalian systems and clinical studies in human demonstrated that EGb761 exhibits a range of biochemical and pharmacological effects, which include: vasoregulation, cognition enhancement, and alleviating stress [17,62]. In human studies, available data have confirmed the clinical efficacy of EGb761 in primary degenerative dementia of Alzheimer's type [33,34,41]. Although the evidence supporting EGb761 enhancement of learning and longevity in healthy animals and humans is inconclusive [16,68], there are sufficient data to support the view that the extract has neuroprotective properties [60].

EGb761 has been shown to protect animals from effects of hypoxia [27], ischemia [47], and to reduce the behavioral deficits resulting from brain injury [2]. Several lines of evidence indicate that EGb761 counters the effects of stress and aging [53], at least in part, through an increase in the density of α -2 adrenoreceptors [25] and serotonergic (5-HT_{1A}) receptors [24] in brain tissue. In isolated synaptosomes from mice cerebral cortex, EGb761 treatment elevated 5-HT uptake [52], which is down regulated in degenerated brain. Choline uptake was also increased in EGb761-treated hippocampal synaptosomes [31]. Choline is a precursor for biosynthesis of the neurotransmitter acetylcholine, which plays a crucial role in memory and learning processes. Loss of basal forebrain cholinergic neurons has been directly related to AD [3].

Synergistically, EGb761 may protect brain by affecting cerebral blood flow and energy metabolism in experimental animals. EGb761 treatment resulted in an increased blood flow, elevated levels of ATP and glucose in rats [28,34], and reduced levels of free fatty acids in hippocampus of rats under seizure conditions [54]. Animal behavioral studies by Cohen-Salmon et al., suggest that long-term treatment with EGb761 reduces some stress-induced behavioral changes in old mice [43]. The authors have observed an effect of EGb761 on improved performance of a learning task in aged mice chronically treated with EGb761. Furthermore, they found a statistically significant hippocampal structure changes in post mortem histological analysis of these mice, particularly in the mossy fibers in CA3 region of the hippocampus [14]. At a recent Meeting of Society for Neuroscience, two groups of investigators, working on different systems, reported on a possible role of EGb761 in neuroregeneration [12, 19].

Ginkgolide B (BN52021), a component of EGb761, may protect neurons by being an antagonist of a receptor for the platelet-activating factor (PAF) [60]. PAF is an alkylphospholipid produced by a variety of cells; it is one of the most potent lipid mediators known [61]. PAF induces neuronal apoptosis, glutamate release and transcriptional activation following excitotoxic challenge [5]. PAF concentration is known to increase in the brain during trauma [27], which results in an increase in free intracellular Ca²⁺ concentration [30]. Specific PAF receptors have been identified in the neurons, localized to intracellular membranes, synaptic endings and microglia [37]. Bazan and colleagues have demonstrated that ginkgolide B (BN52021) has impressive neuroprotective properties [5]. Nevertheless, based on

the in vivo animal experiments mentioned above, it is unclear to what extent EGb761 exerts its function directly on central nervous system (CNS).

In vitro experiments indicate that EGb761 has direct effects on CNS. EGb761 attenuated neuronal cell death induced by serum deprivation and staurosporine in cultured chick embryonic neurons and neonatal rat hippocampus [1] and by amyloid β in primary cultured hippocampal neurons [4]. It protected neurons against oxidative stress induced by hydrogen peroxide in dissociated rat cerebellar neurons [40,42], against cytotoxicity induced by calcium channel blockers in rat cortical neurons [72] and by glutamate in HT-4 neuronal cells [29]. In accord with its role as an anti-oxidant, EGb761 suppressed the reactive oxygen species (ROS) formation in an ischemia model of cerebellar neurons [42]. Large body of analysis has revealed that, in a cellular or in vitro system EGb761 interacts either directly or indirectly with nearly all ROS of biological significance. It not only meets all of the criteria that are required for characterizing a substance as an anti-oxidant [17], but also could be the most efficacious antioxidant.

Mitochondria both generate and detoxify ROS. Thus, imbalance of this compromise plays a key role in chronic diseases [46]. EGb761 may prevent mitochondrial aging by its free-radical scavenger effect. In cultured cells, EGb761 stimulates mitochondrial gene expression of the respiratory-chain enzyme complex I, which is decreased in several neurodegenerative disease [10]. The same author recently suggested that EGb761 inhibits excitotoxic neuronal death by antagonizing the effect of glycine [11]. Janssens and colleagues, using isolated mitochondria from rats, demonstrated that EGb761 protects mitochondria against ischemia-induced oxidative stress [26]. Mitochondrial DNA (mtDNA) is especially susceptible to oxidative damage and mutations, which, in turn, contributes to many degenerative diseases [65]. Sastre et al. recently showed that oral administration of EGb761 to rats for 3 month was able to prevent the age-associated oxidative damage to mtDNA, oxidation of mitochondrial glutathione, as well as changes in mitochondrial morphology and function in brain and liver [55,56]. These results suggest that EGb761 prevents mitochondrial aging by attenuating the chronic oxidative stress associated with this process.

3. Possible mechanisms of neuroprotection

Considerable theory and some evidence suggest that oxidative imbalance (stress), mitochondrial bioenerget-

ics defects, excitatory neurotoxicity, calcium cytotoxicity and trophic factor deficiencies may contribute to cell death in neurodegenerative diseases [36,38,44]. Current consensus is that two broad mechanisms, oxidative stress and excessive activation of glutamate receptors, are converging and represent sequential as well as simultaneous processes that provide a final common pathway for cell vulnerability in the brain [15]. Being a mixture, EGb761 may exert its neuroprotection in an interactive, synergistic way, e.g., by inducing the protective pathway against oxidative stress, and at the same time inhibiting the apoptotic machinery. The specific pathways are depicted in Fig. 1.

Several laboratories demonstrating EGb761 neuroprotection against oxidative stress, excitotoxicity, and neurotoxicity have emphasized possible anti-oxidant properties of EGb761 as one of its mechanisms of action [49]. EGb761 may act not only as a radical scavenger: processes downstream of the anti-oxidative effect of EGb761 may also be involved in the inhibition of apoptosis. For example, EGb761 inhibits lipid peroxidation and increases the levels of superoxide dismutase (SOD), an endogenous antioxidant enzyme system [63]. Even the anti-apoptotic action of EGb761 may be mediated by its antioxidative capacity. Also, as a PAF antagonist [7], EGb761 suppresses the PAF-induced generation of reactive oxygen species [6].

Among numerous theories raised to explain neurodegenerative Alzheimer's diseases, the "amyloid beta ($A\beta$)-induced free radical-mediated neurotoxicity" hypothesis [69] is especially attractive because it provides a rationale for intervention. That is, administration of "exogenous antioxidant" such as EGb761 may slow the progress of the disease. According to this theory, free radicals may be the link between $A\beta$ -induced cellular damage and cytotoxicity in AD. This hypothesis is based on two observations: 1) aggregation of $A\beta$, the main constituent in AD plaques, is toxic to neurons [70], thus may initiate and promote neurodegeneration, and 2) aggregated $A\beta$ protein itself spontaneously generates more reactive oxygen radicals that can damage the cells [21,22]. Additional experimental evidence supporting this hypothesis includes: the antioxidant vitamin E protected cortical synaptosomal membranes and cultured hippocampal neurons from $A\beta$ -induced toxicity [8] and EGb761 protected hippocampal neurons against amyloid β -induced cell death [4]. However, experimental results from other laboratories argue against this theory. The $A\beta$ aggregation-induced free radical release [22] has been described as an experimental artifact [20]. Mason and

coworker suggested that amyloid β peptide (25–35) in fact has an antioxidative activity: it inhibited lipid peroxidation in a liposome membrane [66]. If free radicals mediated the $A\beta$ -induced toxicity, an antioxidant would block the effect. However, Pike et al. observed that antioxidants did not protect against $A\beta$ -induced neurotoxicity in primary cultured rat embryonic cells [48]. Yao and coworkers also demonstrated that ginkgolides prevented the $A\beta$ -induced increase of reactive oxygen species (ROS), but that did not rescue the cells from $A\beta$ -induced apoptosis in cultured PC12 cells [71]. They concluded that the free radicals and lipid peroxidation do not mediate $A\beta$ -induced neuronal cell death.

The current understanding is that in vivo oxidative status is a dynamic balance between pro-oxidant and oxidant defenses and is yet difficult to determine [58]. Even the pathological lesion of AD, senile plaques and neurofibrillary tangles, may play an important aspect in defense, i.e. protection of neurons from oxidation [46]. The discrepancy among those results obtained from in vitro studies could be due to different models of oxidative stress, different chemicals used, different treatment paradigms and the use of different markers for oxidation. Given that multiple markers were found in vivo to be related to neuronal death in AD [57,59], Perry et al emphasized the importance of appreciating the full extent of protection from oxidants [46]. It is possible that EGb761, a mixture of many compounds, but not the antioxidants ginkgolides and Vitamin E alone, could prevent the $A\beta$ -induced toxicity by its polyvalent action including potential effects on $A\beta$ conformation. Even a direct physical interference with the process of $A\beta$ aggregation cannot be presently excluded as a potential mechanism of action of EGb761. Further, the experiments mentioned above were conducted in vitro, on either artificial membrane or cultured cells. Unequivocal evidence could, perhaps, be obtained by studies using a transgenic animal model of AD [51].

Several laboratories have reported anti-apoptosis effects of EGb761 [1,40,42,72], but the mechanism is still unknown. Recent advances in our understanding of the processes that control apoptosis include the following discoveries [67]: 1) Fas receptor mediates initial events leading to apoptosis, 2) the release of proteins, particularly cytochrome c from mitochondria, triggers activation of caspases, a family of proteolytic enzymes, and 3) activation of caspase 3 is critical for the initiation and execution of apoptosis. None of the components of the apoptosis pathways have yet been demonstrated to contribute to EGb761 neuroprotective action. How-

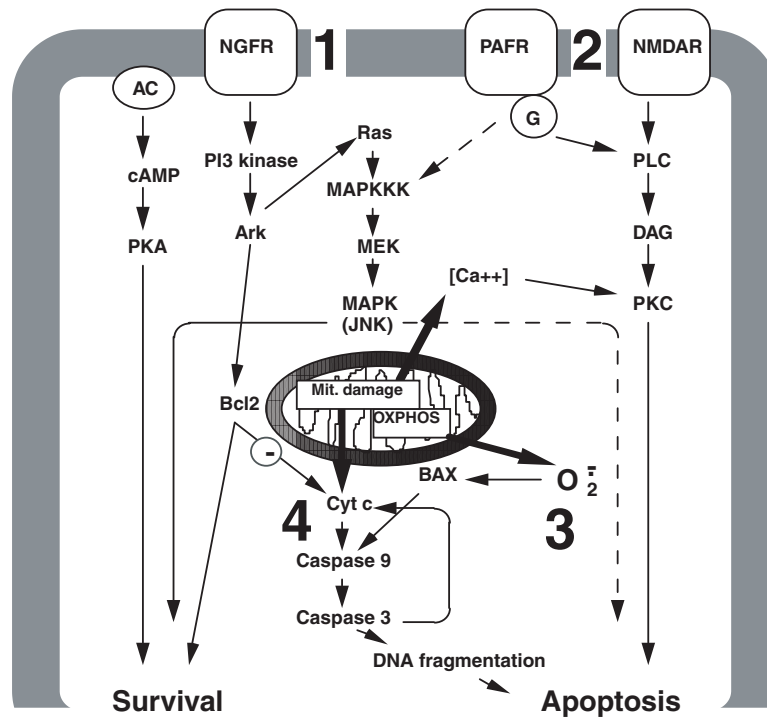


Fig. 1. Potential mechanisms whereby EGb761 may exert neuroprotective effects. 1) EGb761 may stimulate NGFR (nerve growth factor receptor)/PI3 kinase-mediated cell survival pathway for its protective effect against apoptosis induced by serum deprivation. 2) EGb761 may inhibit PAFR (platelet activating factor receptor)- and/or NMDAR (NMDA receptor)-mediated apoptosis pathway, involving $G\alpha$ (G protein α subunit), PKC (protein kinase C) and MAPK cascade (MAPKKK \rightarrow MEK \rightarrow MAP kinase). 3) As an antioxidant, EGb761 may function as a free radical scavenger, and an inhibitor of free radical production. Thus, protect cells from oxidative damage. 4) EGb761 may prevent cellular aging by targeting at mitochondria cell death machinery. Both the pro-apoptotic factor Bax and the anti-apoptotic factor Bcl2 are located on mitochondria (Mit). Under multiple stimuli, BAX activates caspases-3 by releasing cytochrome c (Cyt c) from mitochondria. Caspase 3 then act on intracellular substrates to execute the cell death program. Other pathways include PKA (protein kinase A)-mediated survival pathway and Ras/MAPK cascade-mediated cell proliferation/differentiation pathway; AC: adenylyl cyclase; OXPHOS, oxidative phosphorylation; $[Ca^{2+}]_i$: intracellular calcium concentration. The dashed lines indicate alternative pathways. A circle with “-” represents an inhibitory pathway.

ever, as a PAF antagonist, EGb761 may mediate neuroprotection, at least partially, by a signal transduction pathway similar to those used by nerve growth factor (NGF). PAF is a potent phospholipid mediator, which elicits a diverse array of biological actions by interacting with G protein-coupled PAF receptor (PAFR) [9]. PAFR expression is associated with neuronal apoptosis [5]. PAF enhances excitatory synaptic transmission in the hippocampus by activating pre-synaptic PAF receptors [13]. In most PAF-responsive cells, binding of PAF to its receptor (PAFR) is accompanied by activation of phosphoinositide phospholipase C, leading to the production of the lipid second messenger and activation of protein kinase C. There are some similarities between PAFR activation and apoptosis induced by serum deprivation. Identification of the growth factor signal transduction pathways induced by EGb761

would be of obvious interest, for peptide growth factors have been implicated in the process of brain development, neuronal plasticity, survival and repair [23].

4. Implications for Alzheimer's disease

The key feature of neurodegenerative disorders is neuronal cell loss, which leads to a loss of brain tissue and function, which in turn, may manifest as loss of memory in Alzheimer's disease. While the etiology of Alzheimer's diseases remains unknown, a protective therapy based on the identification of the mechanism responsible for cell death may provide an intervention that slows down or stops progressive neurodegeneration. Application of neuroprotection as the treatment of AD has received increasing attention. EGb761, stimu-

lating the growth factor-mediated cell survival pathway, would have profound therapeutic implications since the proteinaceous trophic factors, such as NGF, do not cross the blood-brain barrier as easily as EGb761 does. Although it is not universally agreed whether the cell death in AD is apoptotic or necrotic in nature [45], inhibition of the apoptotic component of neuronal death may be another promising new therapeutic strategy [64]. EGb761 has numerous properties, which theoretically should be beneficial in treatment of AD. Its multiple beneficial actions, including increased blood flow, antioxidant activity, inhibition of platelet activating factor and nitric oxide, and neuroprotective activity, suggest that EGb761 could be of major therapeutic value in the treatment of AD. Although that has yet to be proven, it has already been determined in double-blind, randomized, placebo-controlled clinical trials, that EGb761 has no more adverse effects than placebo treatment [33,34]. Pre-clinical evidence suggests an important role for antioxidant treatment in protection against free radical-induced neuronal death. This has not yet been confirmed in clinical trials, but available data are promising [50] and warrant further investigation. If antioxidants such as vitamin E and EGb761 are proven efficacious in additional AD trials, the simplicity of treatment, ease of access, and low cost render these agents attractive as treatments to delay or slow down the effects of this devastating disorder. It may be that a combination of vitamin E (which is effective at free-radical scavenging) and EGb761 (which might be most effective at preventing further free-radical production) would be a rational way to use antioxidant therapy in aging and AD. Side effects of high dose use of vitamin E might be attenuated in combined therapy with EGb761.

5. Conclusion

The simplicity of treatment, ease of access, and low cost made Ginkgo biloba extract one of the most popular complementary and alternative medicines for prevention and treatment of primary neurodegenerative dementias. In vivo and in vitro experimental evidence indicates its neuroprotective effects by a "polyvalent" action, which may be mediated, at least in part, by anti-oxidative and anti-apoptotic mechanisms. We hypothesize that such polyvalent action may have most efficacious function in preventing oxidative damage in the cells, presumable by synergistically regulating oxidative status/balance. Understanding the mechanisms

of Ginkgo biloba neuroprotection may bring about new possibilities for therapies that exploit multi-leveled, synergistic action against neurodegeneration. Combination therapies, using Ginkgo biloba extracts, represent new avenues for treating neurodegenerative diseases such as Alzheimer's disease and ameliorate the consequence of neuronal degeneration in aging.

Acknowledgments

The author thanks Drs. Peter Butko, Margaret McDonald, and Witold K. Surewicz for helpful discussions and for reading the manuscript.

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