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Review

Effect of a phytopharmaceutical medicine, *Ginko biloba* extract 761, in an animal model of Parkinson's disease: Therapeutic perspectives

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ABSTRACT

Ginkgo Biloba extract 761 (EGb 761) is a patented and well-defined mixture of active compounds extracted from *Ginkgo biloba* leaves. This extract contains two main groups of active compounds, flavonoids (24%) and terpenoids (6%). EGb 761 is used clinically to treat dementia and vaso-occlusive and cochleovestibular disorders. This extract has neuroprotective effects, exerted probably by means of its antioxidant function. Parkinson's disease (PD) is a neurodegenerative disorder that affects 2% of the population older than 60 y. It produces a progressive loss of dopaminergic neurons and depletion of dopamine (DA), leading to movement impairment. The production of reactive oxygen species, which act as mediators of oxidative damage, is linked to PD. This disease is routinely treated with the DA precursor, L-3,4-dihydroxyphenylalanine. However, this produces severe side effects, and its neurotoxic properties can be due to a free radical production. Thus, administration of antioxidant drugs might be used to prevent neuronal death produced by oxidative mechanisms. The use of synthetic antioxidants has decreased because of their suspected activity as carcinogenic promoters. We describe the studies related to the antioxidant effect of EGb 761 in an animal model of PD. It has been shown that EGb761 can provide a neuroprotective/neurorecovery effect against the damage to midbrain DA neurons in an animal model of PD. EGb 761 also has been found to lessen the impairment of locomotion, correlating with an increase of DA and other morphologic and biochemical parameters related to its antioxidant effect in an animal model of PD. These studies suggest it as an alternative in the future treatment of PD.

Introduction

Currently, there is worldwide interest in finding new and safe antioxidants from natural resources to prevent the oxidative deterioration of living cells. The use of synthetic antioxidants has decreased because of their suspected activity as carcinogenic promoters. We introduce a natural extract with antioxidant action, *Gingko biloba* extract 761 (EGb 761), as a potential medicine in the treatment of Parkinson's disease (PD). EGb 761 is a well-defined mixture of active compounds extracted from *G. biloba* leaves. EGb 761 is prepared as a dry powder and contains two groups of major substances, flavonoid (24%) and terpenoid (6%) fractions. It has been proposed that EGb 761 has beneficial neuroprotective effects, probably by means of its antioxidant action, which involves the scavenging of free radicals [1–4]. EGb 761 has been used clinically for the treatment of diseases related to free radical production leading to oxidative stress, such as cerebrovascular insufficiency, degenerative dementia, and neurosensory disorders [5].

The cause of the degeneration and death of the dopaminergic neurons of the nigrostriatal pathway in PD is unclear, but a large body of evidence has indicated that oxidative stress, by free radical production, and the generation of reactive oxygen species (ROS) as events occurring selectively in the substantia nigra pars compacta (SNpc) of parkinsonian brains [6,7] play an important role in the pathogenesis of PD. Human postmortem studies have also suggested that oxidative damage to lipids, proteins, and DNA occur in the SNpc of patients with PD [8,9]. Oxidative stress can

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induce neuronal damage and modulate intracellular signaling, ultimately leading to neuronal death by apoptosis or necrosis [10]. Apoptotic and non-apoptotic pathways, which may simultaneously occur in patients with PD, may be induced through the activation of common pathways upstream of mitochondrion-dependent cell death [11]. Thus, antioxidants have been studied for their effectiveness in decreasing these deleterious effects and neuronal death.

The purpose of this review was to discuss the potential use of EGb 761 in the prevention and treatment of PD, mainly focusing on its antioxidant action against oxidative stress. We explain the findings on the neuroprotective/neurorecovery effect of EGb 761 on oxidative stress induced by the neurotoxin 1-methyl-4-pheynyl-1,2,3,6-tetrahydropyridine (MPTP), the best animal model of PD. In addition, we discuss the use of EGb 761 as a potential alternative treatment of PD.

EGb 761 composition

Ginkgo biloba is one of the oldest living tree species and has been described as a living fossil, and its beneficial effects were known 5000 y ago in traditional Chinese medicine. The study of the biological activities of EGb 761, a standardized extract of *G. biloba* with a well-defined mixture developed more than 20 y ago, has gained great popularity in European countries and the USA. EGb 761 has been standardized to ensure the consistency of its composition and a reliable, safe, efficacy profile [12].

The EGb 761 extract is a well-defined mixture of active compounds extracted from *G. biloba* leaves according to a standardized procedure [13]. EGb 761 is prepared as a dry powder and contains two main groups of active compounds, flavonoids (~24%) and terpenoids (~6%) [13]. The flavonoid fraction is composed primarily of three flavonols: quercetin, kaempferol, and isorhamnetin, which are linked to a sugar. The flavonoids consist of three benzene rings: benzene ring (A) is condensed with a 6-membered ring (C), which in the 2 position carries a phenyl ring (B) as a substituent. The 6-member ring condensed with the benzene ring is an a-pyrone (flavonols and flavonones) or its dihydro derivative (flavanols and flavanones). The position of the benzenoid substituent divides the flavonoid class into flavonoids (2 position) and isoflavonoids (3 position) [12]. These flavonols exhibit antioxidant actions, are free radical scavengers and enzyme inhibitors, and have cation-chelator, antiallergic, anti-inflammatory, antiproliferative, antiviral, and anticarcinogenic effects [12].

The terpenoid fraction (~6%) is composed of ginkgolides (~3.1%) and bilobalides (~2.9%), which are found exclusively in the *G. biloba* tree. 1) Ginkgolides are diterpenes (20 carbon atoms arranged into six 5-member rings), which are responsible for the bitter taste of EGb 761. Five ginkgolides have been identified as constituents of EGb 761 (ginkgolides A, B, C, J, and M) [12]. 2) Bilobalides are sesquiterpenes (15 carbon atoms arranged into six 5-member rings) [12].

Other components of EGb 761 are organic acids (~5–10%), which include 3-methoxy-4-hydroxybenzoic acid (vanillic acid), 4-hydroxybenzoic acid (*p*-hydroxybenzoic acid), 3.4-dihydroxybenzoic acid (protocatechuic acid), acetic acid, shikimic acid, 6-hydroxykynurenic acid, kynurenic acid, and ascorbic acid. These substances give an acidic character to the extract, thereby increasing the water solubility of its flavonoids and terpene constituents, which are not water soluble [12]. EGb 761 also contains proanthocyanidins (>0.5%), defined as flavonoid-based polymers [12] and less than 5 ppm of alkylphenols and, in particular, ginkgolic acids because these can produce toxic effects [12].

Pharmacokinetic of EGb 761

The pharmacokinetic properties of EGb 761 are difficult to specify because of the complexity of the extract. Studies in healthy human volunteers have shown that flavonoids are absorbed in the small intestine [14]. The peak of plasma concentrations is 2 to 3 h, with an elimination half-life of 2 to 4 h. Complete elimination occurs 24 h after ingestion of the extract [12]. With regard to the terpenoid fraction, it has been reported that the absolute bioavailabilities of ginkgolides A and B are practically complete, regardless of the dosage, whereas that of gingkolide C is very low [12]. Ginkgolides A and B reach peak plasma concentrations 1.4 to 2 h after administration and have half-lives of 3.9 and 7 h, respectively. The bioavailability of bilobalide is 72%, with a half-life of 3.2 h after oral administration. The ginkgolides are eliminated with urine, and ginkgolides A and B are generally unchanged [12].

Toxicology of EGb 761

The EGb 761 extract is well tolerated and does not influence primary hemostasis at pharmacologically active doses [15]. This extract is also safe, as shown in studies of subchronic and chronic toxicity in rodents. Animal toxicologic data have reported the oral 50% lethal dose in mice is more than 9600 mg/kg, which is 2100 times greater than the recommended daily dose [15]. Most clinical studies with EGb 761 have shown good or very good tolerability (50–600 mg/d) in acute and long-term trials [16]. The adverse effects, e.g., nausea, vomiting, headaches, and dizziness, are rare, mild, transient, and reversible. Blood pressure, heart rate, and cholesterol and triacylglycerol levels seem to be unaltered with the intake of EGb 761 at the dose as mentioned earlier [15,16]. No mutagenic, carcinogenic, teratogenic, or embryotoxic effects have been demonstrated for this *G. biloba* extract.

Dosage of EGb 761

The EGb 761 extract has a wide therapeutic window. The proper efficacious dose can differ according to the situation, but pharmacologic experiments have concluded that 50 to 100 mg · kg^{-1} · d^{-1} is usually active (although 10 mg/kg has exerted beneficial effects in some models) [17]. In humans, the most common daily dose used is 120 mg (usually EGb 761 40 mg taken three times daily) or 240 mg. The clinical effects in central nervous system diseases normally emerge after about 4 wk of treatment [18]. However, further investigation should be performed for its potential use in the treatment of PD.

Protective mechanisms of EGb 761

Anti-inflammatory effects of EGb 761

Inflammation has been implicated in various neurodegenerative alterations. In particular, substantial evidence has suggested the role of platelet-activating factor (PAF) as a regulator of cytokines in inflammatory responses. This factor has been implicated in neuroinflammatory processes in PD [19]. EGb 761 has been demonstrated to have anti-inflammatory effects. These effects may be attributed to the combined actions of its ginkgolide and flavonoid constituents. Ginkgolides display very specific and potent antagonist effects against PAF [20]. The intracerebroventricular

administration of ginkgolide B (BN52021) in rats has been shown to significantly attenuate the PAF-induced increase in cerebrospinal fluid peptide leukotriene levels [21]. Ginkgolide B also has been shown to decrease the PAF-induced production of eicosanoid and thromboxane B in a fetal rat brain [22] and completely block the PAF-induced decrease of cell viability in SH-SY5Y cells [23]. In addition to its PAF-antagonizing activity, the inhibitory effects of ginkgolides A and B on proinflammatory cytokine tumor necrosis factor-α and interleukin-1 production in lipopolysaccharide-stimulated rat microglial cultures have been observed [24].

Antioxidant properties of EGb 761

The EGb 761 extract exhibits important antioxidant properties [25]. EGb 761 can scavenge many ROS, such as hydroxyl radical ($OH^{\cdot}$), peroxyl and oxoferryl radical, and superoxide ($O_2^{\cdot-}$) anions, and nitric oxide, in vitro [2,25]. The antioxidant action of EGb 761 is due mainly to the flavonoid fraction, which scavenges $O_2^{\cdot-}$ anions and $OH^{\cdot}$ and peroxyl radicals [25,26]. Flavonoids also prevent lipid peroxidation (LP) in membranes, particularly owing to their ability to interact with and penetrate the lipid bilayers [27]. The terpenoid fraction has been found to have antioxidant effects in a neurodegenerative model [28]. Terpenes may potentiate the antioxidant action of flavonoids in EGb 761.

The EGb 761 extract also may exert antioxidant actions indirectly; for example, it decreases peroxide generation in the brain and liver [29] and increases superoxide dismutase (SOD) and catalase activities in the hippocampus, striatum, and substantia nigra [30]. EGb 761 also decreases the age-dependent increase in cerebral monoamine oxidase B (MAO-B) activity, which is another source of ROS [31], and it has been suggested that EGb 761 protects the mitochondrial respiratory chain [32] against free radical production.

The antioxidant action of EGb 761 makes it an excellent candidate as an antiaging drug, including in PD. For instance, rats treated for a long period with EGb 761 live significantly longer than controls and exhibit better cognitive performance [33]. EGb 761 has strong antioxidant properties and thus may modulate the signal transduction pathways that are sensitive to oxidative stress. For example, EGb 761 may modulate the response to a wide variety of extracellular stimuli through the inhibition of transcription factors activated by oxidative stress. Of particular importance are those signal transduction pathways related to apoptosis. Therefore, the antioxidant actions of EGb 761 may contribute to the prevention of cell death, particularly apoptosis [3].

Antiapoptotic effect of EGb 761

The antiapoptotic actions of EGb 761 are multifunctional and may act synergistically on multiple intracellular signaling pathways involved in apoptosis. As possible mechanisms underlying its antiapoptotic action, EGb 761 may maintain the integrity of the mitochondrial membrane that prevents cytochrome-c release [34–36], thereby blocking the formation of the apoptosome and the apoptotic caspase cascade [35,36]. EGb 761 also increases the transcription of antiapoptotic Bcl-2–like protein and inhibits the proapoptotic factor Bax [37–39], attenuates the transcription of proapoptotic caspase-7 and -8 [35,36], and inactivates the proapoptotic c-Jun N-terminal kinase, thereby "turning off" downstream target c-Jun [23]. In addition, EGb 761 inhibits the cleavage of the key effector protease caspase-3, thus blocking the execution of apoptosis and preventing nuclear DNA fragmentation, the molecular hallmark of apoptosis [23,34,40]. The antiapoptotic effects of EGb 761 have been observed in 6-hydroxydopamine (6-OHDA) [41], paraquat [42], and MPTP [43] animal models of PD.

Oxidative stress in PD

The cause of the degeneration of dopaminergic neurons in the SNpc in PD is unclear, but there is evidence that oxidative stress, by free radical production, plays an important role in the process [44]. Oxidative stress produces prominent neuronal injury. It is caused by the chemical imbalance between production of ROS and the decrease of antioxidants (agents and enzymes) [45]. An overproduction of ROS has been found in PD [46], where oxidative stress occurs continually. Neurons are considered highly susceptible to oxidative stress because of the low levels of antioxidants.

The ROS are a family of highly reactive small molecules that contain oxygen (O_2), including O_2 radicals and non-radical derivatives of O_2. Free radicals are defined as atoms, molecules, or ions that have unpaired electrons, resulting in an unstable species [47]. Mitochondria are one of the main intracellular sources of ROS [48]. Mitochondrial $O_2^{\cdot-}$ radical formation is generated during oxidative phosphorylation, which interacts with O_2 [49]. $O_2^{\cdot-}$ can be transformed into other forms of ROS, such as hydrogen peroxide (H_2O_2), by the enzyme SOD or can react with nitric oxide and be converted into peroxynitrite [47]. Furthermore, H_2O_2 can be transformed into the highly reactive $OH^{\cdot}$ by Fenton reactions involving transition metal ions [50]. Indeed, $OH^{\cdot}$ is so reactive with different targets, including DNA, RNA, lipids, and proteins, that it is considered the most potent of all ROS.

However, organisms have developed various defense mechanisms to protect themselves against damage by ROS. In most mammalian cells, this includes antioxidant enzymes such as SOD, catalase, and glutathione peroxidase (GPx); the latter two convert H_2O_2 to water [50]. In neurons, it has been shown that SOD and GPx are expressed in larger quantities than catalase. However, the low levels of catalase increase the risk of overexposure to H_2O_2 and other ROS that derive from it [51]. Other antioxidant molecules present in the neurons include vitamin E, melatonin, and glutathione.

In particular, the brain is rich in polyunsaturated fatty acids, which make it highly susceptible to oxidative stress by LP, a chain reaction that results in numerous degradation products. Several studies of postmortem brain tissues of patients with PD have suggested that oxidative stress is involved in the degeneration of dopaminergic neurons [46]. In PD, the concentration of polyunsaturated fatty acids in the substantia nigra is decreased, whereas malondialdehyde, a marker of LP, is increased [52]. Lipid oxidation in PD is also increased as indicated by an increase of 4-hydroxy-2-nonenal, a lipophilic product of the peroxidation of the membrane-bound arachidonic acid [53]. Levels of markers of oxidative damage to proteins have been reported to be significantly increased in postmortem samples of substantia nigra in PD brains [54]. DNA also has been shown to exhibit oxidant damage by ROS in postmortem samples of substantia nigra from PD brains [55]. The content of common deletions in mitochondrial DNA, which can be caused by oxidative stress, is increased in the substantia nigra in patients with PD [56]. In particular, SOD is often regarded as the first line of defense against ROS. In PD brains, no changes have been found in Cu, Zn-SOD (cytosolic isoform) activity, but an increase in Mn-SOD (mitochondrial isoform) activity has been reported

[57]. Mn-SOD is highly inducible in response to an excess of ROS. Therefore, the increase in Mn-SOD in PD suggests that the mitochondrial compartment in PD is the site of increased ROS production. In contrast to SOD, catalase and GPx activities are decreased in PD brains [58].

Monoamine oxidase exists in two isoforms, A and B, and is the major intracellular enzyme that metabolizes dopamine (DA) in the central nervous system (Fig. 1). High oxidation of DA produced by MAO-B in PD could lead to the production of H_2O_2, a toxic O_2-derived molecule able to produce free radicals that can damage nigrostriatal neurons [59]. MAO-B activity is increased in patients with PD [60] and MAO-B inhibitors have a potential neuroprotective effect in neurodegenerative disease [61].

In addition, apoptosis may play an important role in PD [62]. Therefore, it is important to prevent the process using pharmacologic agents that can scavenge free radicals and decrease the apoptosis.

Parkinson's disease is routinely treated by the administration of the DA precursor, L-3,4-dihydroxyphenylalanine (L-DOPA), resulting in an increased synthesis of the neurotransmitter DA in the brain. However, this promotes the degeneration of nigral dopaminergic neurons by causing additional oxidative stress from auto-oxidation products and increasing the DA content and turnover [63,64]. L-DOPA treatment has secondary effects, some of them severe. Therefore, the search for new therapeutic possibilities is of considerable interest. In this context, plant extracts are an alternative for neuroprotection. In particular, EGb 761 is an antioxidant and neuroprotective agent in a variety of medical conditions.

Experimental model of PD induced by MPTP/1-methyl-4-phenylpyridinium ion

The neurotoxin MPTP is regarded as the best available experimental model (Fig. 2) of the neurochemical sequelae of PD [65]. This is a dopaminergic neurotoxin that, when administered to non-human primates and mice, causes nigral cell loss and symptoms similar to those in PD [65]. Its active metabolite, 1-methyl-4-phenylpyridinium ion (MPP^+), is taken up in dopaminergic terminals by the plasma-membrane DA transporter and is accumulated in mitochondria [66]. The high concentration of MPP^+ inside the mitochondria blocks complex I activity in the respiratory chain [66]. MPTP oxidation to MPP^+ by MAO-B in the brain generates free radical production [67]. Incubation of MPP^+ with mitochondrial enzymes induces free radical production [68], and the increase in free radicals can further inhibit the function of complex I. MPTP- and MPP^+-induced toxicities are linked, in part, to oxidative stress by the production of ROS [69]. Further supporting evidence includes the observation that pretreatment of mice with diethyldithiocarbamate, a SOD inhibitor, increases MPTP-induced neurotoxicity [70]. In particular, transgenic mice overexpressing human Cu,Zn-SOD have shown increased SOD activity and greater resistance to MPTP [71]. With regard to the oxidative stress hypothesis, an increase of LP has been reported, a process dependent on free radical overproduction, and a consequence of MPP^+ administration to mice [72]. In line with these findings, several antioxidants (radical scavengers) have been reported to protect against MPP^+- and MPTP-induced neurotoxicities [73]. Therefore, it appears reasonable to propose that exogenous antioxidants such as EGb

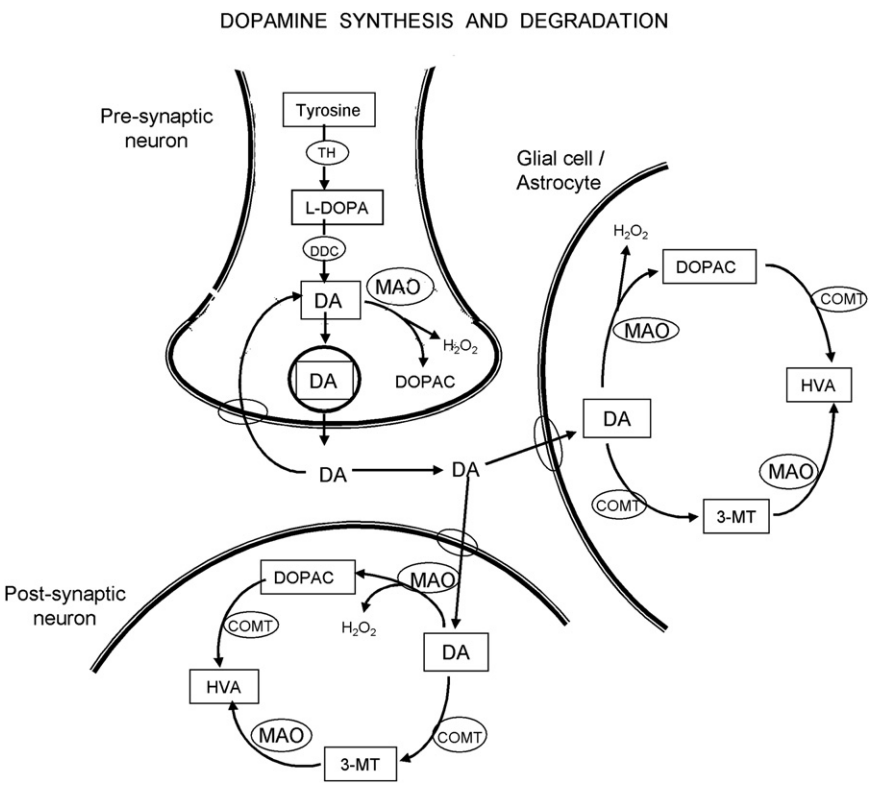

Fig. 1. Key steps in the synthesis and degradation of DA. Tyrosine is converted to L-DOPA by TH. DA present in a free state within the presynaptic terminal can be degraded by the enzyme MAO and can be inactivated by the enzyme COMT. 3-MT, 3-methoxytyramine; COMT, catechol-*O*-methyltransferase; DA, dopamine; DDC, 3,4-di-hydroxy-phenylalanine decarboxylase; DOPAC, dihydroxyphenylacetic acid; H_2O_2, hydrogen peroxide; HVA, homovanillic acid; L-DOPA, L-3,4-di-hydroxy-phenylalanine; MAO, monoamine oxidase; TH, tyrosine hydroxylase.

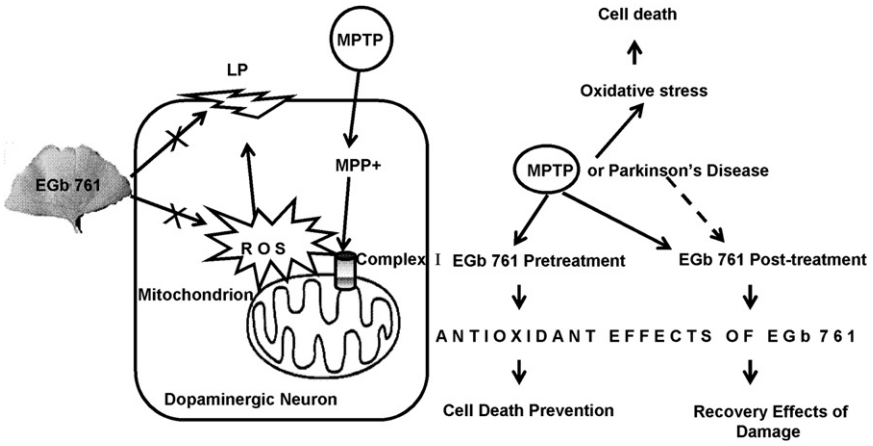

Fig. 2. Schematic representation of the neuropreventive and neurorecovery effects of EGb 761 in Parkinson's disease or its animal model. MPP^+ (the active metabolite of MPTP) inside the mitochondria blocks complex I activity in the respiratory chain, which induces oxidative stress by the production of ROS. An overproduction of ROS may induce LP and cell death. In Parkinson's disease, oxidative stress is one of the main mechanisms that cause cell death. However, EGb 761 pretreatment prevents the cell death induced by MPTP. Further, EGb 761 post-treatment reverses the damage produced by MPTP-induced neurodegeneration. The two neuroprotective effects of EGb 761 are mainly by means of its antioxidant effects through the blockage of oxidative stress. Studies in experimental models have suggested that EGb 761 may be a therapeutic alternative for patients with Parkinson's disease (dashed arrow). EGb 761, *Ginkgo biloba* extract 761; LP, lipid peroxidation; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ROS, reactive oxygen species.

761 may be effective in decreasing oxidative stress damage in PD and MPTP/MPP^+ neurotoxicity.

EGb 761 pretreatment prevents oxidative stress damage in an animal model of PD

The administration of MPP^+ to rodents increases the production of free radicals and LP [72]. An excess generation of free radicals, leading to LP, has been proposed to play an important role in the damage of dopaminergic striatal neurons induced by MPP^+. The antioxidant effect of pretreatment with EGb 761 on the oxidative stress induced by MPP^+ neurotoxicity in the striatum has been reported [17] and might be caused by the putative action of EGb 761 as a free radical scavenger and its antioxidant properties (Fig. 2). EGb 761 may block the LP produced by MPP^+ probably caused by scavenging OH^- radicals and $O_2^{\cdot-}$ anions [25] or prevent the formation of lipid peroxyl radicals [2], the major chain-propagation step in LP, resulting in the indirect inhibition of LP. EGb 761 has been reported to decrease LP in a free radical-generating system [74], prevent apoptosis induced by OH^- radicals [3], and protect against oxidative stress in mitochondria [29].

Pretreatment with EGb 761 has been shown to decrease LP gradually in a dose-dependent manner [75] in an animal model of PD induced with 6-OHDA. Moreover, EGb 761 pretreatment has been found to have a neuroprotective effect in a paraquat-induced model of PD through the maintenance of potential mitochondrial membrane stability [76].

In addition, it is important to consider that EGb 761 has been shown to protect neurons against oxidative stress induced by several types of ROS, such as $O_2^{\cdot-}$ anion, H_2O_2, and OH^- radicals. In particular, EGb 761 pretreatment has been shown to provide dopaminergic protection (32%) against the DA-depleting effect of MPP^+ neurotoxicity [17]. Additional studies using 6-OHDA [77] have shown that EGb 761 pretreatment improves locomotor activity, which correlates with increased DA levels and neuronal preservation in the substantia nigra.

Dopamine synthesis might be altered by free radicals, producing the peroxidation of membrane lipids and an alteration of DA uptake [78]. These mechanisms could participate in MPP^+ neurotoxicity because free radicals are produced during its neurotoxicity. In addition, decreased synaptosomal DA uptake has been shown to occur under peroxidative conditions and to be prevented by EGb 761 [79].

The protective effect of different *G. biloba* extracts against oxidative stress in MPTP neurotoxicity has also been reported. Yang et al. [43] showed that pretreatment with a *G. biloba* extract 1) prevented dopaminergic neurotoxicity, 2) decreased SOD activity, 3) decreased the oxidative stress produced by MPTP, and 4) prevented the apoptosis induced by MPP^+, the stable metabolite of MPTP. However, it is important to consider that these extracts have compositions different from EGb 761.

EGb 761 pretreatment regulates MAO in an animal model of PD

The biotransformation of MPTP to MPP^+ is performed by MAO-B in the brain, and this event produces free radicals [67]. Moreover, MPP^+ is actively accumulated in mitochondria, where it produces free radicals, a process that can inhibit the complex I of mitochondria. These findings suggest that neuronal death occurs by different events, such as a loss of energy production and oxidative stress by the production of ROS by MPP^+ and/or MAO activity. In addition, MAO-B inhibitors have been implicated in neuroprotection in PD and MPTP/MPP^+ neurotoxicity [80].

The MPP^+ neurotoxicity is prevented by treatment with the MAO inhibitor pargyline. EGb 761 has been reported to protect against MPTP neurotoxicity, and the inhibition of MAO produced by EGb 761 may be related to its neuroprotective effect [81] because MAO-B is required for the biotransformation of MPTP to MPP^+. In particular, EGb 761 produces reversible inhibition of the two MAO isoforms in the central nervous system [31].

The neuroprotection of EGb 761 against MPP^+ (the stable metabolite of MPTP, administered directly in the brain) has been reported to be produced most probably by a downregulation of MAO-B activity [4] rather than a simple inhibition, preventing the MAO-B activity from increasing above a certain level, as previously reported [31]. The mechanism proposed is supported

because, in this case, EGb 761 did not interact peripherally with the metabolism of MPTP to MPP^+ or with its brain access, because MPP^+ was administered locally to the brain.

The regulating effect of EGb 761 pretreatment on MAO-B during MPP^+ neurotoxicity may be related to its decrease of free radical formation [82], because we reported that EGb 761 pretreatment blocks MPP^+-induced LP [17]. It is important to point out that an increased oxidation of DA by MAO-B may involve the production of H_2O_2, a toxic O_2-derived molecule able to induce free radical formation, which could potentially damage nigrostriatal neurons.

The increase of MAO-B activity precedes the DA depletory effect of MPP^+ neurotoxicity. EGb 761 pretreatment in MPP^+ administration has been shown to increase DA metabolism-related markers such as tyrosine hydroxylase activity and DA levels [82]. These findings are supported by the demonstrated effect of EGb 761 on DA neuronal metabolism [83]. The MAO and tyrosine hydroxylase activities might be regulated through independent mechanisms produced by EGb 761 in MPP^+ neurotoxicity.

L-deprenyl (selegiline) is currently the only MAO-B inhibitor available for therapeutic use in PD. However, its metabolism generates amphetamine and met-amphetamine [84]. Thus, it appears to be important to develop agents with MAO-regulating activities that are not metabolized to products with amphetamine-like actions; EGb 761 is an important alternative in this respect.

EGb 761 post-treatment reverses damage produced in an animal model of PD

We previously reported a neurorecovery effect of EGb 761 by administrating the natural extract after the last MPTP administration, when neurodegenerative mechanisms are activated (Fig. 2). These data demonstrated that EGb 761 can provide effective protection against the damage to midbrain DA neurons arising from the neurotoxic effects of MPTP *in vivo*. This protection might be due, in part, to stabilization of the DA uptake system. EGb 761 has been shown to prevent the alteration of the neuronal DA uptake system [79]. This effect was also observed in vivo, where the dopaminergic neurons were protected against the neurotoxin MPTP when mice received EGb 761 2 wk before the neurotoxin was infused peripherally through an osmotic mini-pump over 7 d [83].

Lipid peroxidation may damage the membrane fluidity of the cell by damaging the receptors and ion channels in the cell membrane [85], which may result in calcium influx and cause cell death. EGb 761 prevents the modification of the membrane fluidity induced by a pro-oxidant system, ascorbic acid/Fe^{2+} [74]. We also reported the presence of an increased LP at 18 d after the last injection of MPTP [4]. However, EGb 761 blocked LP in the MPTP-treated groups, suggesting that the neurorecovery effect of EGb 761 is due, at least in part, to its antioxidant properties [25,29].

The role of EGb 761 as a free radical scavenger in MPTP neurotoxicity is supported by our previous findings [4]. Mn-SOD activity was increased in response to MPTP neurotoxicity and decreased in the "MPTP + EGb 761" group. We propose that the EGb 761 protection against MPTP neurotoxicity is due primarily to the scavenging of $O_2^{\cdot-}$ free radicals and preventing an increase in Mn-SOD activity. This suggests that EGb 761 inhibits $O_2^{\cdot-}$ production in MPTP neurotoxicity [4].

These results are in line with previous reports showing that EGb 761 regulates other antioxidant enzymes such as GPx and glutathione reductase [86,87]. Other *G. biloba* extracts have been reported to restore the activities of glutathione-dependent enzymes, catalase, and SOD in the striatum after 6-OHDA administration [75]. EGb 761 may possess neurotrophic and/or neuritogenic properties that improve functional recovery after neuronal injury [88].

The locomotor deficits observed in MPTP neurotoxicity were improved after exposure to EGb 761, probably because of a partial protection of striatal DA levels. These findings would appear to be further strengthened by the normalization of denervation-related supersensitivity of DA D2 receptors in the striatum by EGb 761. This normalization of DA D2 receptors has been reported for a *G. biloba* extract in 6-OHDA–induced Parkinsonism [75]. It is well documented that the denervation-related upregulation of these receptors is a compensatory mechanism for a DA deficit [89]. EGb 761 has been reported to increase the transcripts encoding brain proteins related to neuronal and synaptic plasticity [90], which may protect against MPTP neurotoxicity.

Locomotor effects are not just a functional improvement, because it has been clearly demonstrated that EGb 761 treatment reverses the neurodegeneration of nigrostriatal dopaminergic neurons produced by MPTP neurotoxicity. This is supported by the larger numbers of SNpc tyrosine hydroxylase-positive neuronal bodies and striatal fibers [4].

This recovery may be due to EGb 761 activating transcription genes that encode peptides important in cellular growth and function [90]. These events may contribute to the restoration or regeneration of neurons after MPTP neurotoxicity. The studies supporting our findings have proposed that EGb 761 ameliorates the neurodegeneration associated with aging [5] and this may occur in MPTP/MPP^+ neurotoxicity.

A protective effect of other *G. biloba* extracts in MPTP neurotoxicity is supported by improvement of locomotor activity [43]. Ahmad et al. [75] also showed that administration of a *G. biloba* extract improved locomotor activity by increasing the density of tyrosine hydroxylase-positive neuronal bodies in the substantia nigra against 6-OHDA neurotoxicity.

Cao et al. [91] studied the neuroprotective effect of a *G. biloba* extract in combination with L-DOPA after 6-OHDA administration. Their results showed that a combination of a *G. biloba* extract with L-DOPA decreases apoptosis and decreases the impairment of locomotor activity compared with L-DOPA alone.

Conclusion

The EGb 761 is a standardized extract of a well-defined complex mixture of compounds and is a non-toxic drug. This phytopharmaceutical medicine exerts potent neuropreventive/neurorecovery effects in the MPTP/MPP^+ model of PD by its antioxidant effects. Further, its antiapoptotic effect may be another mechanism of neuroprotection.

In particular, current drugs such as L-DOPA provide only symptomatic relief and for a limited duration. This might be related to additional oxidative stress from the auto-oxidation products of L-DOPA. EGb 761, which can decrease oxidative stress and ameliorate neurodegeneration in MPTP neurotoxicity, appears to be a good candidate to effectively slow the progressive neurodegeneration in PD. One important issue when searching for drug therapies for patients with PD is the potential side effects during or after long-term administration. In this regard, EGb 761 has a very impressive clinical safety record and has been introduced in the clinic for the treatment of vascular dementia and Alzheimer's disease (mixed dementia), tinnitus, and vertigo. This

makes it an excellent candidate for further investigation of its usefulness in the treatment of PD. EGb 761 studies have clearly shown its potential in the future treatment of PD.

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