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GINKGO BILOBA AND NEURODEGENERATIVE DISORDERS

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TABLE OF CONTENTS

1. ABSTRACT

The *Gingko biloba* extract EGb 761 has been the subject of many studies which confirm its usefulness for the prevention and treatment of neurodegenerative pathologies. These studies have focused on: a) the probable mechanisms of action that are involved in these disorders (including non-specific mechanisms implicated in diverse neurodegenerative disorders, particularly oxidative stress, or specific mechanisms such as those associated with beta-amyloid in Alzheimer's disease) and the processes of neuronal death; b) available animal models, and c) healthy individuals or those suffering from mild cognitive impairment or Alzheimer's disease. This data must be completed, particularly with regard to new knowledge about the pathogenesis of these disorders. Ambitious interventional studies are underway and may provide new evidence regarding the effect of EGb 761 in preventing Alzheimer's disease in humans. Positive findings would be particularly interesting since this drug is very safe to use.

2. INTRODUCTION

The *Ginkgo biloba* tree is phylogenetically unique today, an originality that is reflected in its remarkable biochemical composition. Several molecules (ginkgolides and bilobalide) exist only in this tree. The flavonoids, however, belong to a vast family of plant origin, but appear in an unusual form in the tree: rather than standard components such as quercetin or kaempferol, they are combined with sugars (flavonol-O-glycosides). In traditional Chinese medicine, an infusion of boiled leaves, mixed with other preparations, is used to treat heart and lung diseases. However, the uses of *Gingko biloba* extracts in Western medicine owe little to these traditional applications (1). The *Gingko biloba* extract EGb 761 (Tanakan) is standardized to contain 24% flavonoids and 6% terpene lactones (3.1% ginkgolides and 2.9% bilobalide). The vast majority of laboratory and clinical studies involve this particular extract. EGb 761 was first marketed in France in 1975 and then in other countries. It has been approved by government agencies as an ethical drug and is reimbursed by government-backed health insurance programs in France, Germany, Spain, Belgium, Netherlands, Switzerland, Portugal, Russia, Czech Republic, Romania, Austria and Turkey, as well as Argentina, Brazil, China, Thailand, etc. In the United States and Japan, on the other hand, it is considered a food supplement. EGb 761 is indicated in diverse circulatory, cerebral and neurosensory deficits. Several studies demonstrate its interest in age-associated cognitive disorders, and Alzheimer's disease (AD) in particular. The drug classification developed by the French Caisse Nationale d'Assurance Maladie (see 2), in common with the WHO classification, the German certification (3), and the Belgian authorities list EGb 761, along with cholinesterase inhibitors, as treatments for dementia.

Over the past few decades, EGb 761 and to a lesser extent other extracts of *Ginkgo biloba*, have been the focus of many studies. This work involves various areas, but particularly the effect of these products on the nervous system and neurodegenerative pathologies. What is remarkable about this body of work is that it involves various levels of organization in living beings: molecular, cellular, tissue, whole animal, human, behavioral, etc. (4).

3. EFFECT ON NON-SPECIFIC MECHANISMS OF NEURODEGENERATIVE PATHOLOGIES: ANTIOXIDANT EFFECT AND PROTECTION AGAINST NEURONAL DEATH

Neurodegenerative disorders can be described as pathologies of neuronal death, particularly via apoptosis (5)

and the result of two mechanisms (6). One of the mechanisms is non-specific, associated with senescence, and no doubt mainly involves oxidative stress. The other mechanism is specific to each pathology, and associated with the type of protein whose abnormal aggregation is implicated in the disorder (beta-amyloid [Abeta] in AD, alpha-synuclein in Parkinson's disease, tau in fronto-temporal dementia, etc.). This double mechanism leads to two therapeutic strategies: the use of antioxidants that can act on all neurodegenerative pathologies, as well as specific approaches, the most characteristic of which is immunization assays (6).

There are now many arguments that implicate oxidative stress in neurodegenerative disorders, particularly AD (7-8). These arguments are both theoretical (the importance of oxidative metabolism in neurons) and experimental [the toxicity of Abeta and other aggregated peptides in neurodegenerative pathologies associated with oxidative stress; the presence of biomarkers for oxidative damage in the cerebrospinal fluid of patients suffering from AD or mild cognitive impairment (MCI; 9); the benefits of a diet rich in antioxidants (10-11)], etc. The involvement of reactive oxygen species (ROS) in the pathogenesis is associated with other non-specific mechanisms involved in the inflammatory cascade leading to cell death: production of tumor necrosis factor-alpha (TNFalpha), interleukin-1beta (IL-1beta), etc.

Many experiments have shown that EGb 761 exerts an antioxidant effect (12), and that its flavone glycosides are among the best radical scavengers both *in vitro* and *in vivo* (13). The free radical scavenger effect of EGb 761 has even been demonstrated *in vivo* in humans (14). In addition, work on the nematode *Caenorhabditis elegans* has shown that the oxidative stress resistance provided by EGb 761 is associated with increased lifespan (15). This effect is particularly interesting since EGb 761 can act as a scavenger for many types of free radicals (superoxide, lipoperoxide, hydrogen peroxide, peroxinitrite, etc.). This has also been demonstrated more directly in models of neurodegenerative pathologies (16).

In addition to this protective action against ROS, EGb 761 has an inhibitory effect on various mediators in the inflammatory reaction involved in the non-specific pathway leading to pathogenesis, particularly TNFalpha (17-21; although ref. 22 noted increased transcription of TNFalpha) and various interleukins, especially IL-1beta (18-19, 23-24). Most of these effects were demonstrated on traditional models of vascular smooth muscle, endothelial cells, neutrophils, T-cells, etc. but some of these studies involve cerebral targets, including inflammation following focal ischemic injury in rats (24) or microglia (18-19), a category of neuroglia thought to play a role in some neurodegenerative diseases in the central nervous system.

EGb 761 protects against neuronal death induced by various neurotoxic agents, including peroxynitrite (25), glutamate (26), hydrogen peroxide (27-28), various agents that induce oxidative stress (29), MPP+ and paraquat (30), as well as Abeta (31-32; see further down), etc. This effect is crucial since, regardless of the hypothesis chosen to explain the pathogenesis of diverse neurodegenerative disorders, the important conclusion is that they all result in neuronal death (5). Thus, one of the most obvious goals of any therapy must be to reduce or stop this neuronal death.

At the cellular level, the antioxidant and antiapoptotic effects (32-35) of EGb 761 are associated with several effects on mitochondria, which are both sources and targets of ROS. EGb 761 protects mitochondria during the aging process (36), and protects them against anoxia/reoxygenation-induced injury (37). Furthermore, it attenuates mitochondrion-initiated apoptosis and decreases the activity of caspase-3, a key enzyme in the apoptosis cell-signaling cascade (32). Its component bilobalide has been shown to increase the levels of mtDNA-encoded cytochrome oxidase subunit III and protein levels (a key enzyme in the protection against cell death in the hippocampus after ischemia (38), protect complex I and III (39-40), increase the respiratory control ratio of mitochondria (41) and inhibit hypoxia-induced decreases in ATP content in endothelial cells (40). This action at the mitochondrial level plays an essential role in the biological effect of EGb 761, and is associated with its antioxidant action. But the two effects are not entirely the same since flavonoids are the main agents responsible for the free radical scavenging effect, while bilobalide is implicated in many other mitochondrial effects (42). This dual action is also implicated in the antiapoptotic effect of EGb 761, which is clearly associated with its flavonoid fraction (43), but also – at least in part - with bilobalide (44).

Moreover, EGb 761 has the special characteristic of activating natural antioxidant defenses. This could appear as a paradoxical effect since, as a product that can scavenge free radicals, EGb 761 could in theory decrease these defenses. In fact, it activates gene or protein expression of endogenous antioxidants such as heme oxygenase-1 (45-46), manganese superoxide dismutase, glutathione, gamma-glutamylcysteine synthetase, which is the rate-controlling enzyme for glutathione synthesis (47), and subunit 1 of mitochondrial NADH dehydrogenase, which is the most complex of mitochondrial respiratory enzymes, and plays a key role in energy metabolism and oxidative phosphorylation (if its activity is inhibited by more than 25%, energy metabolism is severely altered; ref. 48). EGb 761 also protects vitamin E (49), and can activate transcription factor NFkappaB, at least in models of cell death. This transcription factor is very sensitive to the pool of free radicals that are present in the cell. When the free radical concentration is elevated, NFkappaB is activated and increases the expression of several protective genes. By reducing the quantity of free radicals, antioxidants should decrease activation of this transcription factor. But Dembele et al. (50) showed that EGb 761 activates NFkappaB (the same holds true for vitamin E; see ref. 51) and that this activation is associated with protecting the Neuro2A cell line against apoptosis induced by high concentrations of H_2O_2.

Other neuroprotective mechanisms have been identified in addition to free radical scavenging. EGb 761

has been shown to be effective for some of these, e.g. by inducing heat shock protein HSP70 (52), thought by some to play a protective role in Parkinson's disease (53) and in AD (54). EGb 761 also increases the expression of the antiapoptotic agent Bcl-2 (22). In addition, *Ginkgo biloba* extract increases the expression of neuron-specific enolase and S-100 protein mRNA in newborn rat brain after hypoxic-ischemic damage (55).

4. EFFECT ON THE SPECIFIC MECHANISM INVOLVED IN ALZHEIMER'S DISEASE: THE ACTION OF ABETA

Today, there are many arguments implicating Abeta in the development of AD: it is a major component of senile plaques, mutations in its precursor protein (APP) can cause AD, overexpression of the gene containing these deleterious mutations in transgenic mice results in a pathology that resembles AD, Abeta production is increased in various genetic forms of AD, even those that are not due to mutations in the APP gene, etc. (56). The deleterious effect of Abeta can be decreased in several ways. One way is to inhibit its formation by inhibiting the enzymes that cut the Abeta peptide segment from APP (beta- and gamma-secretases) or to increase the action of alpha-secretase, which cuts within the Abeta segment. Other ways include inhibiting the formation of small Abeta oligomers, inhibiting its aggregation, inhibiting its toxicity or facilitating its clearance. EGb 761 has an effect on several of these mechanisms. Both *in vivo* and *in vitro*, it increases the activity of alpha-secretase (57), thereby decreasing the formation of Abeta, but also increasing the formation of soluble APPs segments which are neurotrophic. It decreases Abeta aggregation (32,58) and inhibits its toxicity (31), particularly that linked to beta-amyloid-derived diffusible neurotoxic ligands (59).

This activity involving interference with Abeta metabolism can be studied on Tg2576 transgenic mouse model that overexpress the so-called Swedish mutation in APP. Stackman et al. (60) used this model to demonstrate the ability of a *Gingko biloba* extract to restore normal memory function. Curiously, this effect was not associated with any significant decrease in the amyloid load in the brains of these mice. An even more surprising finding was that concentrations of carbonylated proteins, which normally reflect oxidative damage, were increased in treated animals. These results disagree with anything seen in other models. They also disagree with the work of Backsai et al. (61), which shows that EGb 761 decreases oxidation signaling in the brains of these transgenic mice. But they fit quite well with the recent discovery of Sung et al. (62) who found that the other well known antioxidant vitamin E suppresses brain lipid peroxidation and reduces Abeta levels and amyloid plaques in the Tg2576 mice only when administered early during evolution. Unfortunately this last work provide no behavioral data. If, according to Stackman et al. (60) *Ginkgo biloba* extract restore normal memory without a decrease of amyloid burden, we will maybe have to revisit the amyloid hypothesis in such a way that the toxicity of amyloid could be not related to the plaque but rather to the intracellular Abeta.

EGb 761 also exerts an effect on other biochemical mechanisms that seem to be associated with AD. Specifically, it strongly increases expression of the transthyretin gene *in vivo* (63), which is known for its ability to trap the Abeta peptide, and as such is considered to be protective. This is shown by the fact that Tg2576 mice with elevated transthyretin levels do not show signs of neurodegeneration (64). This transthyretin-inducing effect may be shared with other neuroprotective substances such as omega-3 fatty acids from fish oil and nicotine (65). EGb 761 also increases the production of apolipoprotein E (66), which may play a neuroprotective role for neurons, and protects it against oxidation (16, 67). Remember that in addition to the senile plaques that are mainly composed of Abeta, AD is characterized by intracellular neurofibrillary tangles (NFT) made up of hyperphosphorylated microtubule-associated tau. Watanabe et al. (63) have shown that EGb 761 administered as a dietary supplement in mice upregulated tau mRNA 3- to 4-fold (nicotine and tacrine exert the same effect). EGb 761 also increases mRNA levels for neuronal tyrosine/threonine phosphatase 1 (upregulated 7-fold). This is an interesting property since phosphatase 1 might dephosphorylate tau and prevent the formation of NFT.

We should also note that, at the anatomical level, many observations have shown the beneficial effect of EGb 761 at protecting part of the brain that is highly implicated in aging and AD: the hippocampus. Barkats et al. (68) showed that chronic treatment with EGb 761 preserves hippocampal synapses in older animals.

5. OTHER NEUROCHEMICAL, CELLULAR OR METABOLIC EFFECTS OF EGb 761

EGb 761 exerts many other effects, particularly at the neurochemical level, which may be involved in its neuroprotective action. It exerts an effect on the uptake of choline (69), dopamine (70), serotonin, glutamate, glycine (71) and monoamine oxidase (72). It seems that the flavonoid fraction is implicated in the modulation of dopamine transporter protein expression (73). In addition to these *in vitro* effects, EGb 761 exerts *in vivo* effects such as reactivation of the noradrenergic system during aging (74-75), restoration of serotoninergic neurotransmission in stressed animals (76), a regulatory effect on monoamine oxidase activity during aging and stress (76), etc.

EGb 761 reduces glutamate-associated excitotoxicity by non-competitive antagonism of glutamate receptors (77-78). One of its components (bilobalide) was almost as potent as bicuculline and picrotoxin as an antagonist at recombinant (alpha)(1)(beta)(2)(gamma)(2L) $GABA_A$ receptors (79). Ginkgolides also inhibit $GABA_A$ receptors. They are also selective and potent antagonists of the glycine receptor (80). The inhibitory effect on GABA-ergic inhibition in the hippocampus (81) could be involved in its "stimulant-like" effects (82), as well as on the enhancement of long term potentiation (LTP) in older animals (83).

EGb 761 and its flavonoid fraction modulate estrogen receptor beta (ERbeta) protein expression in neuronal PC12 cells (73). *Ginkgo biloba* extract normalizes stress-elevated alterations in brain cathecholamines, serotonin and plasma corticosterone levels (84).

It also affects ischemia-induced phospholipid degradation, decreases production of fatty acids produced by the degradation of arachidonic acid (85), inhibits phospholipase A2 activity (86), and accelerates reincorporation into brain lysophospholipids (87).

It inhibits phosphodiesterase activity leading to increased cAMP levels (88), which may explain its vascular effect, but may also partly explain the memory improvement seen in animal models. Furthermore, it inhibits protein kinase C, which may explain its protective effect against cerebral ischemia (89). In addition, it reduces circulating glucocorticoid levels caused by inhibition of the peripheral benzodiazepine receptor (90) and down-regulates hippocampal glucocorticoid type II receptors (91).

Moreover, it promotes neurogenesis (92-93) and affects situation-specific cerebral metabolism by slowing it in undamaged subjects (94), but compensating for deficient metabolism in case of injury (95). Its effect on synaptic plasticity also seems to be adapted to homeostasis since it is expressed in older mice but not in young animals (83).

6. PROTECTIVE EFFECTS *IN VIVO*

EGb 761 has been shown to exert a protective effect in many models of neurodegenerative pathologies. This is the case for AD in Tg2576 transgenic mice (60) and in *Caenorhabditis elegans* (96), for Parkinson's disease in animals treated with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (70, 72, 97), and for amyotrophic lateral sclerosis in mice carrying a mutation in copper/zinc superoxide dismutase (98). EGb 761 also protects gerbils and rats in models of cerebral ischemia, particularly neuronal destruction in the hippocampus (99-102). It was also shown to be protective in an experimental head injury model using rats (103). In addition, it abolishes cognitive decline seen in transgenic mice that overexpress growth hormone with elevated and progressively increasing free radical processes in the brain that strongly correlate with reduced survivorship (104).

Also, EGb 761 not only protects the hippocampus during aging (68), but also facilitates synaptic plasticity in the perforant pathway (which projects to the hippocampal dentate gyrus and plays an important role in the formation of associative memory) (105).

7. COGNITIVE EFFECTS IN ANIMALS

Work by Stackman et al. (60) on Tg2576 transgenic mice clearly shows the effect of *Ginkgo biloba* extract on the memory function of these animals, which are models of AD. But the positive effects of EGb 761 on memory function, including in stress situations, or in older subjects (106-107) have been demonstrated in many experiments involving mice, rats and even chicks (108) and while using various tests and paradigms, including conventional passive and active avoidance (106, 109), operant conditioning paradigm (110), T-maze discrimination learning task (111), eight-arm radial maze paradigms (107), Morris water maze (60, 112), scopolamine-induced amnesia (113-114), learned helplessness (115), and olfactory learning ability (114). Most of these studies involve chronic treatment with EGb 761 (107, 116-117), but some have involved acute treatment (113). Most of the studies highlight the fact that this positive effect concerns long term memory (108), but some also found an effect on short term memory (118) and many involve older animals (106-107, 111, 119). It is also interesting to note that the effect depends on genetic factors, as shown by a study involving several aged inbred mouse strains, with a clearer effect in animals showing the most damage due to aging (111). Moreover, it is remarkable that EGb 761 enhances LTP (an increase in synaptic efficacy which is a memory-associated pathway) exclusively in aged mice (83), an effect which could be related to the attenuation of GABA-mediated inhibitory input by bilobalide (81) or to the fact that EGb 761 can reverse membrane fluidity in aged mice (109).

It seems that EGb 761 exerts a regulatory effect on some cognitive abilities, particularly decision-making ability in aged or stressed subjects (117). Thus, it demonstrates an anti-stress effect that differs from those of conventional antidepressants and anxiolitics (115, 120) which may in part be due to the action of its ginkgolide constituents on decreasing adrenal glucocorticoid synthesis (90).

8. COGNITIVE EFFECTS IN HEALTHY HUMANS AND THOSE SUFFERING FROM MCI OR AD

Over the last three decades, EGb 761 has been tested in many situations involving healthy subjects, aged subjects with benign disorders and patients suffering from senile dementia, particularly in the case of AD. This work has demonstrated several of its effects. EGb 761 has a positive effect on the dual coding test (121) and on short-term memory (122-123), acts synergistically with a memory training method (124), and affects working memory, psychomotor performance and executive process (125-126). It also has a potent alpha-enhancing effect on the EEG related to a "vigilance-stimulating" effect (127-129).

Several *Ginkgo biloba* extracts have shown a positive effect on the cognitive abilities of normal subjects, and particularly with the use of EGb 761 (130), although this action was not demonstrated by Solomon et al. (131). Nonetheless, we should note that the neuropsychological tests differ from one study to another, and that there are currently no standard procedures for the study of the improvement in cognitive functions of healthy adults. According to Ihl (132), "existing studies [on EGb 761] can be seen as pioneer work in an area without systematic research guidelines." Therefore the data must be interpreted with caution, even though, taken together, it highlights the positive effect of this substance in normal subjects (132), and particularly on cognitive attention functions. In

addition to these data on psychometric tests, Cieza et al. (133) found that EGb 761 improves self-estimated mental health and self-estimated quality of life of healthy volunteers. The fact that EGb 761 appeared to positively affect mental functioning of healthy people has to be interpreted cautiously because it is certainly not obvious that a drug can improve normal cognition. One explanation could be that some of the apparently normal elderly are in fact not perfectly healthy. The other explanation is that it is really possible to improve normal cognition and memory.

Whatever, the main goal of medicine is to correct health decline, in this case MCI and dementia. When compared to a placebo, EGb 761 significantly improved some cognitive parameters (digit copying sub-test of the Kendrick battery, median speed of response on a computerized version of a classification task) in subjects suffering from MCI after 24 weeks of treatment (134). More recently, Ercoli et al. (135) showed that 6 months of treatment significantly improved verbal memory (as compared to a placebo) in subjects with age-associated memory impairment. The magnitude of cognitive improvement correlated significantly with resting lateral temporal metabolism (although overall, the treated and untreated groups did not differ significantly with regard to modifications in cerebral metabolism).

EGb 761 also exerts beneficial effects on attention, memory and functioning in patients with multiple sclerosis (136). EGb 761 has been used in several studies on AD. It showed a statistically significant effect on ADAS-Cog and other cognitive scales in several clinical trials, particularly the study by Le Bars et al. (137) (202 patients, daily dose: 120 mg; evaluation at 12, 26 and 52 weeks). Another study (138) involving several types of dementias found no significant effect (daily doses: 160 mg or 240 mg, 24 weeks of treatment, 214 very heterogenous demented patients with AD or vascular dementia), but meta-analysis by several authors including the investigator of this negative study, and taking into account 33 published trials, led to an overall conclusion in favor of the effect of this substance (139). Dementia studies on patient samples with a high prevalence of BPSD (Behavioral and Psychological Symptoms of Dementia) suggest that that EGb 761 is of particular interest in this group since it improves both the patient's cognitive ability and behavioral and psychological symptoms (140). Differences between the various studies on the cognitive effects of EGb 761 suggest that we should perform a more detailed study of the neuropsychological tests used. The available data show that tests used to evaluate attention ability (particularly the Pace Auditory Serial Addition Test or PASAT) and frontal lobe functions are most sensitive to the action of EGb 761. Thus, it would be more interesting to identify the basic cognitive functions modulated by EGb 761 rather than limiting ourselves to the use of overall scales such as the ADAS-Cog.

9. PREVENTIVE EFFECTS

The effect of EGb 761 was studied using the Epidos cohort from 1992 to 1999, involving 7,598 French women in good health and older than age 75, in order to evaluate the risk factors for fracture of the femoral neck. The 1,462 women of the Toulouse site were then regularly monitored and nearly half of them (n=714) underwent prospective longitudinal evaluation of any cognitive disorders and evolution toward dependency and/or dementia. After including potential confounding factors in a multivariate analysis, women with dementia were found to have had less continuous exposure to vasodilators, including EGb 761 (odds ratio: 0.31 – CI = 95% p = 0.018). If EGb 761 treatment is isolated from other vasodilators, the results are comparable (odds ratio = 0.38 – CI = 95%) but not statistically significant, probably due to insufficient population size (141). This demonstration of a preventive effect could appears as a surprise since there is a strong bias against the drug in this study: many women of the Epidos cohort were probably taking EGb 761 because they had memory problems which could be a sign of the beginning of AD (such a bias does not exist in the case of epidemiological studies involving anti-inflammatory drugs or estrogens). That means that the preventive effect of EGb 761 could be very important.

10. INTERVENTIONAL STUDIES

The Epidos study strongly suggests an effect of EGb 761 (141) on the prevention of AD. Moreover, prevention currently seems to be the most reasonable and effective therapeutic approach, since delaying the onset of AD by five years amounts to reducing the prevalence of this disease by half. In addition to the Epidos study, various epidemiological observations strengthen the hypothesis of a preventive effect by EGb 761, particularly the beneficial preventive effect of a diet rich in antioxidants (10-11), and more specifically flavonoid intake (142) since this is an essential component of EGb 761 (1).

However, this preventive effect cannot be proven by epidemiological observations alone. Large interventional studies are required, involving many individuals who are randomly assigned (and thus without bias) to either treated or control groups and followed for several years. Two of these studies have been undertaken: one in the United States (the GEM study) and the other in France (the GuidAge study). The GEM study (Ginkgo Evaluation of Memory) is being conducted under the auspices of the NIH and coordinated by S. De Kosky (University of Pittsburgh) to examine the potential of EGb 761 to prevent AD in a population of 3,000 subjects over age 75, and treated for 5 years with 240 mg/day of EGb 761 as compared to a placebo (143). The GuidAge study is being performed in France and coordinated by B. Vellas (University of Toulouse) to investigate the potential of a daily dose of EGb 761 at 240mg to prevent AD in patients at risk who are over age 70 and have spontaneously complained to their general practitioner about memory problems. The study includes 2,800 patients, and the results are expected in 2008-2009 (144). It will be very interesting to compare the results of these two studies, which are very similar, except that the GuidAge study includes patients who are at risk in that they have spontaneously reported memory problem. A third study conducted in Oregon under the auspices of the NIH includes 200 subjects over age 85

and aims to assess the prevention of MCI by EGb 761 treatment for 3 years at 240mg/day as compared to a placebo.

11. A HOST OF MECHANISMS

EGb 761 is a complex substance due to its composition, but also its mechanism of action since it acts on many cellular and molecular targets. This complexity reflects a concept that is poorly explained by traditional pharmacology, but is in agreement with current evidence from cellular and molecular biology; namely that cell signaling pathways use ubiquitous signals which are rarely specific for a single signaling pathway, and genes and proteins are expressed in clusters rather than individually. Current tools like DNA arrays, which can be used to study gene expression throughout most of the genome, provide new methods of examining this complexity and it is interesting to note that they are particularly adapted to complex plant extracts like EGb 761 (145). EGb 761 has been shown to be capable of up- or down-regulating many genes both *in vitro* and *in vivo* (63, 146-147).

However, this data must be carefully interpreted for many reasons: a) the *in vitro* effect is not necessarily the same *in vivo*; b) the *in vivo* effects may differ in normal and pathological situations; c) even if these effects are beneficial, they may reflect either activation of beneficial processes or the fact that, since the cell is protected, it has less need to activate a protective process. This reasoning may be used to interpret the observations made by Strayer et al. (148) which demonstrated inhibition of the small heat-shock protein Hsp-16-2 in *Caenorhabditis elegans* by EGb 761. The expression of Hsp-16-2 is induced by the pro-oxidant juglone; since EGb 761 decreases cellular stress resulting from exogenous treatments, it also decreases transcriptional induction of this possibly protective gene. Although gene or protein expression studies are essential, it is clear that they can be difficult to interpret since the expression of protective mechanisms may reflect a beneficial effect of the substance being tested, or conversely a toxic effect that requires a protective response (and vice versa).

12. ACUTE AND CHRONIC EFFECTS

The conventional explanation for the beneficial effect of antioxidants on neurodegenerative pathologies involves their ability to protect against neuronal death. This reasoning takes into account a preventive long term effect, but does not account for immediate actions. But EGb 761 also demonstrates rapid effects, including on cognition in humans, as shown by the studies of Allain et al. (121) on the dual coding test, in addition to work by Hartley et al. (149) which highlighted some effects on cognition in postmenopausal women of one-week treatment with *Ginkgo biloba* on a test of frontal lobe function (rule shifting) and PASAT. It is difficult to explain this type of effect by invoking a neuroprotective mechanism. Acute effects may be explained by action on neurotransmission, while long term effects may be explained by neuroprotection or an effect on synaptic plasticity. An example of this is work by Williams et al. (83) demonstrating the acute effect of EGb 761 on modulating excitability via action on the glutamatergic system and a chronic treatment effect on plasticity and the synaptic network. Another hypothesis which may explain acute effects is based on the numerous effects of EGb 761 on gene expression observed both *in vitro* and *in vivo*. This action is virtually immediate, and is in addition to the protection against cell death for which the effects are only observed after several months (150). It seems probable that antioxidants exert a regulatory action on cells as well as the organism which is not limited to protection against cellular damage, but also is involved in the regulation of metabolism and gene expression. In the case of EGb 761, the result of these complex actions suggests adaptation or regulation rather than a well defined effect; thus, this substance is antiapoptotic in models of neuronal death associated with neurodegenerative pathologies, but pro-apoptotic in models of cancer. It is possible that this adaptive capacity is due to the fact that the substance is a plant extract, i.e. it is not chemically synthesized, but rather the result of natural selection, itself a source of adaptation (151). In contrast to the simple idea that is typically invoked (i.e. antioxidants protect against cellular damage by directly scavenging free radicals), these observations suggest that the effects of antioxidants are in fact extremely complex.

13. HOW EGb 761 CAN BE ADAPTED TO THERAPY FOR ALZHEIMER'S DISEASE AND AGE-RELATED COGNITIVE DISORDERS

Neurodegenerative pathologies are a great challenge for medicine since their prevalence tends to increase due to demographical changes and because drugs available for treatment are lacking. Currently, there are no drugs that can be used to cure AD for example, or even clearly treat the symptoms. In this context, any possible areas for progress should be explored with interest. In this regard, EGb 761 is particularly interesting for the following reasons: a) It is a very good candidate for the prevention of AD, and perhaps other neurodegenerative pathologies; and prevention is, for the time being, the most reasonable and efficient approach given what is currently known; b) It exerts positive symptomatic effects on cognitive parameters; c) It presents no major secondary effects. These are precious advantages for treating a pathology that affects frail and aged subjects. Of course, secondary effects would be acceptable for any therapeutic treatments that could cure the disorder or significantly improve symptoms; but in the absence of such clear progress, treatments must present no further harm to the health of old subjects. In the case of EGb 761, the only concerns observed have involved six cases of spontaneous bleeding which have been reported in patients treated with *Ginkgo biloba* from different extracts. The recent study by Bal dit Sollier (152) demonstrated that no alteration in platelet function or coagulation was in fact induced by EGb 761; d) It has the advantage of acting on several targets of AD, which is indispensable in treating such a multifactorial pathology. It decreases the non-specific processes implicated in neurodegenerescence, such as oxidative damage, inflammatory reaction and cell death by apoptosis. It also

reduces processes which are more specifically associated with the disorder and the involvement of Abeta, whose production it decreases, as well as decreasing aggregation and toxic effects, and facilitating neuronal repair. These multiple effects make it a very good candidate for the treatment of AD, either alone or in combination with other drugs, particularly more specific drugs which will probably be available in the future.

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