Phytochemicals in Alzheimer Disease: The Development of Clinical Trials

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Abstract

Polyphenolics and other phytochemicals may have a role in the prevention or treatment of Alzheimer disease. Targets of therapy in Alzheimer disease include neurotransmitter deficits, beta amyloid neurotoxicity, oxidative damage, and inflammation, to name a few. Challenges to the development of phytochemical and other neuroprotectant therapy in Alzheimer disease include the inability to measure pathology in living patients and the challenge of detecting modification of an indolent disease course. These challenges are partially surmounted by the use of animal models and of biomarkers of disease. This review describes currently available animal models and biomarkers and surveys clinical trials of phytochemical therapies that are recently completed or currently under way. Both animal studies and clinical trials of Ginkgo biloba are described, as well as a trial of Uncaria tomentosa that has not been previously reported.

Keywords: Alzheimer disease, antioxidants, oxidative stress, polyphenolics.

Introduction

Polyphenolics and other phytochemicals may have a role in the prevention or treatment of Alzheimer disease (AD). Abundant epidemiologic evidence indicates that diet plays a role in brain aging and in the pathogenesis of age-associated dementia (Mattson et al., 2002). In addition, treatment trials of Ginkgo biloba extract have been completed (Le Bars et al., 1997; Oken et al., 1998), and prevention studies employing Ginkgo biloba are underway. A pilot study of Uncaria tomentosa in AD has been completed, a small trial of curcumin in AD is underway, and trials of other phytochemicals are being planned. Despite uncertainties regarding the pathophysiology of AD, many clinical trials with phytochemicals are designed with the “amyloid hypothesis” of AD in mind. In vitro screening methods and relatively new animal models of AD oriented to the amyloid hypothesis are influencing the design of many trials. Animal models permit examination of the effect of candidate therapies on brain pathology, an outcome measure that will not be available in human studies until it becomes possible to image AD brain pathology noninvasively (Backsai et al., 2002; Huang et al., 2002). Indirect measurements of pathology are increasingly available, however, and the use of these “biomarkers” in clinical trials is expected to dramatically enhance the interpretation of results. This review will briefly describe the “amyloid hypothesis” of AD, the attendant rationale for polyphenolics and other phytochemicals, an animal model of cerebral amyloidosis used to screen phytochemicals, and the biomarkers currently available for clinical trials in AD. Studies already completed (ginkgo, Uncaria tomentosa) and currently underway (ginkgo for prevention, curcumin) will illustrate the challenges inherent in this approach and will emphasize features of trial design, which may help overcome these hurdles.

Alzheimer disease and the amyloid hypothesis

The pathologic lesions which have received the most attention in AD are extracellular β-amyloid plaques
and intraneuronal neurofibrillary tangles. Neuronal death, synapse loss, selective neurotransmitter depletion, and glial activation are also considered important aspects of the neurodegenerative process, but the “primary” cause is hotly debated. The amyloid hypothesis (Hardy & Selkoe, 2002) holds that β-amyloid, the main component of the plaque, is toxic to neurons and is the driving force behind a neurodegenerative cascade. The amyloid hypothesis is strongly supported by genetic findings in AD and by cell culture studies but is not supported by clinicopathologic studies or by some animal studies.

For example, the genes that have been reproducibly associated with AD in rare families with autosomal dominant AD are intimately involved in β-amyloid metabolism (Price, 1998). In fact, one of the genes with AD-causing mutations in some of these families is the amyloid precursor protein (APP) gene. APP is approximately 700 amino acids in length and may be processed into a number of splice variants, including β-amyloid, which is 40–42 amino acids (Sisodia & St George-Hyslop, 2002). The enzymes responsible for cleavage to β-amyloid are β-secretase and partof -secretase. Another gene associated with AD in some of these families was of unknown function when it was defined by linkage studies and named “presenilin” (Sisodia & St George-Hyslop, 2002). It has since become clear that this gene is associated with increased production of β-amyloid, and it is likely that the presenilin gene codes for part of secretase, an enzyme responsible for cleaving the amyloid precursor protein to form β-amyloid rather than an alternative splice product. Although these instances refer to a small minority of cases of AD, it also appears that the mechanism of the most common risk factor for garden variety AD, apolipoprotein E, present in up to 50% of all cases, may also be mediated by an effect upon β-amyloid (Walker et al., 2000).

The strongest evidence against the amyloid hypothesis is clinicopathologic data. Although there is a weak correlation between amyloid plaque number and dementia severity (Perry et al., 1978), the strongest correlation with dementia severity is synaptic density, followed by neuronal loss and neurofibrillary tangle number, both of which are more highly correlated with cognition than are plaques (Terry et al., 1991). Mechanistic studies do not settle the issue: β-amyloid is toxic to neurons in culture (Cotman et al., 1992), but injections of β-amyloid do not usually produce neurotoxicity in the hippocampus or cortex of intact animals (Harkany et al., 1999), with some notable exceptions (Emre et al., 1992; Geula et al., 1998).

There are also several versions of the amyloid hypothesis, dependent in part on the particular β-amyloid species that is considered toxic: the soluble form, the fibrillar (plaque) form, or a diffusible oligomeric intermediate (Lambert et al., 1998). In any case, the amyloid hypothesis represents one way of conceptualizing the degenerative process in AD and is the basis for therapeutic strategies, which aim to either reduce β-amyloid or ameliorate its neurotoxic effects.

The rationale for using polyphenolics in AD is based on the observations that (1) AD is associated with oxidative brain damage (Montine et al., 2002a), (2) the neurotoxicity of β-amyloid in culture can be attenuated with a variety of antioxidants (Cowman et al., 1992), and (3) polyphenolics have potent antioxidant activity (Galli et al., 2002). The potential therapeutic action of phytochemicals, however, is not limited to antioxidant effects. Some phytochemicals also have anti-inflammatory activity (Liu et al., 2001), which may be relevant in light of inflammatory mechanisms in AD (Akiyama et al., 2000). The rationale for using still other phytochemicals is based on assays of β-amyloid fibril formation (Lu et al., 2002), as inhibition of fibrillogenesis may be valuable for enabling the brain to clear β-amyloid that is not fixed in plaques.

**Transgenic models of AD**

Because aging rodents do not develop the brain pathology of AD, the first report of a transgenic mouse that developed β-amyloid plaques aroused a great deal of excitement (Games et al., 1995). Other models quickly appeared, most of them based on the insertion of a mutant human APP into the mouse genome and characterized by the age-dependent appearance of β-amyloid plaques in the hippocampus and cerebral cortex (Hsiao, 1998). Perhaps the most widely available strain is Tg2576, developed by Dr. Karen Hsiao-Ashe (Hsiao et al., 1996) and used by us and others in the screening of phytochemicals for the treatment of AD. The transgene is a mutant human APP on a prion promoter. The transgene is expressed from the time of birth, but hippocampal and cortical plaques do not appear until about 10 months of age. This strain also develops an impairment in hippocampal-dependent spatial memory that is detectable in the Morris Water Maze, validating this strain as a model of hippocampal dysfunction associated with amyloidosis (Hsiao et al., 1996). This strain also replicates AD pathology in terms of astrocytic activation (Hsiao et al., 1996), microglial activation (Frautschy et al., 1998), neuritic dystrophy (Hsiao, 1998), inflammation (Benzing et al., 1999), and oxidative damage (Smith et al., 1998). On the other hand, this strain has to be considered an incomplete model of AD, as it lacks neuronal death (Irizarry et al., 1997), neurofibrillary tangles, and cholinergic dysfunction (Hernandez et al., 2001).

**Animal studies of phytochemicals for AD**

Modulation of brain aging with complex extracts rich in polyphenolics has been described in the aging of wild-type rodents, with encouraging and important
results (Galli et al., 2002). This discussion, however, will focus on findings in mouse models of AD characterized by cerebral amyloidosis.

**Ginkgo biloba**

Although clinical studies of *Ginkgo biloba* have been inconclusive (Oken et al., 1998), in vitro studies indicate that ginkgo may prevent β-amyloid fibril formation (Luo et al., 2002) and may attenuate β-amyloid neurotoxicity (Bastianetto et al., 2000; Yao et al., 2001). We examined, in the Tg2576 mouse, the effect of chronic *Ginkgo biloba* therapy upon brain levels and consequences of β-amyloid (Stackman, 2001). *Ginkgo biloba* was added to the drinking water of treated mice starting at 8 months of age (before plaques are present), whereas control mice were given tap water. Both drank comparable quantities of water and were examined in the Morris Water Maze at age 14 months, after 6 months of treatment. Both wild-type and transgenic mice were studied in order to distinguish an effect on age-associated cognitive impairment from an effect on β-amyloid–associated impairment. Untreated Tg2576 mice were slower to learn the location of a hidden platform and less able to retain the memory of the location than untreated wild-type mice. Ginkgo-treated Tg2576 mice learned and remembered at a level comparable to wild-type animals (Stackman, 2001). Ginkgo-treated wild-type mice were similar to untreated wild-type animals on the learning task. All groups were similar in the visible platform paradigm, which controls for general sensorimotor function apart from cognition (Table 1).

Mice were then sacrificed at 15 months, an age when β-amyloid plaque pathology is typically well established. Brain levels of β-amyloid were not different in ginkgo-treated compared to untreated animals. Both ELISA measurements and immunohistochemistry revealed similar brain levels of β-amyloid in the two groups. ELISA measurements were performed on both soluble and insoluble fractions of brain homogenate, and separate measurements of β-amyloid 1–40 and 1–42 were performed, with no differences in any measurement (Stackman, 2001). The mechanism of the ginkgo effects on learning remains uncertain, but it is clear that *Ginkgo biloba* did not exert an antiamyloid effect in this treatment paradigm.

**Curcumin**

Cole and colleagues have examined the effect of curcumin, a polyphenolic compound with anti-inflammatory and antioxidant compounds, in Tg2576 mice (Lim et al., 2001). Mice were placed on a curcumin-enriched versus control diet at 10 months of age and maintained on that regimen until sacrifice at age 16 months. The curcumin-treated animals had significantly lower levels of hippocampal β-amyloid and also had diminished oxidative damage as measured by protein carbonyls compared to those on the control diet. The curcumin-treated animals also had lower brain levels of interleukin-1β and less staining for activated microglia, indicating an anti-inflammatory effect of this agent (Lim et al., 2001). Mice in this study were not behaviorally tested (Table 1).

**Uncaria tomentosa**

Extracts of this woody vine, commonly called “cat’s claw,” have been shown to have antioxidant and anti-inflammatory activities in a number of paradigms (Sandoval et al., 2002). The alkaloid components of *Uncaria tomentosa* have also been shown to improve memory function in mice with experimental amnesia (Mohamed et al., 2000). More specific to AD, a proprietary extract of *Uncaria tomentosa*, PTI-00703, potently inhibits β-amyloid fibril formation and even solubilizes pre-formed amyloid fibrils (Snow, 1999). An extract enriched in the fibril-inhibiting factors was also shown to diminish the amyloid plaque burden in an animal model of AD analogous to the Tg2576 strain (Snow, 2001). Measures of inflammation, oxidative damage, and learning were not reported in these animals (Table 1).

**Planning clinical trials of phytochemicals:**

**The status of biomarkers of AD**

It is possible to derive volumes of brain regions of interest from MRI scans, and a number of studies have...
validated brain volumes as markers of AD. For example, hippocampal volumes are smaller in AD subjects than in controls (Laakso et al., 1998). In fact, hippocampal atrophy is detectable even in nondemented subjects who are destined to develop dementia (Kaye et al., 1997). Hippocampal atrophy has also been correlated with AD pathology in several studies (Jack et al., 1999, 2002; Silbert, 2003). The rate of brain atrophy has also been validated as a marker of disease. Comparison of elderly subjects who were destined to develop dementia showed a greater rate of cortical atrophy than subjects who remained stable. Rates of brain atrophy correlate with rates of clinical decline (Fox et al., 1999; Quinn, 2000), and rates of brain atrophy predict the amount of AD pathology at autopsy (Silbert, 2003). The ultimate validation of the “brain atrophy rate” as a useful outcome measure will be the demonstration of an effect of a therapeutic intervention upon rate of brain tissue loss. Ironically, clinical trial designers cannot justify inclusion of these expensive measures until they are validated as sensitive to pharmacologic interventions, and that validation will not take place unless the measurements are included in clinical trials of candidate neuroprotectant agents.

MRI spectroscopy measurements of N-acetyl-aspartate has also been used as a marker of neuronal integrity in clinical studies in AD (Spencer et al., 2003) and may have a role as an outcome measure in clinical trials. A number of methods are currently in development for the specific imaging of β-amyloid plaques in living AD patients (Bacskai et al., 2002; Shoghi-Jadid et al., 2002), but none of these methods is currently available for clinical trials.

Another alternative to imaging plaques directly is to measure body fluid levels of β-amyloid as a surrogate measure. Such measurements are possible but difficult to interpret. Cerebrospinal fluid (CSF) β-amyloid 1–42 distinguishes AD patients from control subjects, but the difference is somewhat counterintuitive. The AD patients, whose brains are full of β-amyloid plaques, have significantly lower levels of β-amyloid 1–42 than age-matched controls (Sjogren et al., 2003). The post facto explanation is that β-amyloid is trapped in the brain in plaques, and the lower CSF levels in patients reflect the diminished amounts available to circulate out of the parenchyma. Serial measurement of CSF β-amyloid suggests that it is relatively stable in mild AD (Kanai et al., 1998; Pirttila et al., 1998), so that changes in the setting of experimental interventions have the potential to reflect inhibition of β-amyloid production, or effects on solubility. Plasma levels of β-amyloid 1–42 do not distinguish AD patients from controls (Tamaoka et al., 1996), probably because plasma levels do not correlate with CSF levels (Mehta et al., 2001), because plasma levels are probably not all derived from brain, and because binding to plasma proteins confounds the measurement (Kuo et al., 1999). The value of plasma β-amyloid as an outcome measure in clinical trials continues to be explored.

Another CSF marker is tau protein, a component of the intracellular neurofibrillary tangle, also of interest as a potential neuronal marker of AD. CSF tau levels are consistently higher in AD patients than in control subjects (Sjogren et al., 2003). Serial levels of CSF tau do not show significant change over time unless the values are corrected for changes in degree of brain atrophy (de Leon et al., 2002). Plasma levels of tau are not informative.

A third CSF marker that is particularly relevant to the antioxidant strategy inherent in trials of polyphenolics is CSF F 2-isoprostan e s (Morrow & Roberts, 1997). These are stable lipid peroxidation products that are increased in concentration in AD brain (Montine et al., 1999b) and CSF (Montine et al., 1999a). F 2 isoprostane levels in postmortem brain are correlated with brain atrophy (Montine et al., 1999b). We also found an inverse correlation between CSF F 2 isoprostanes and total brain volume in living subjects in the Uncaria tomentosa study described below (Fig. 1). CSF isoprostanes may help to distinguish AD from control subjects, especially when combined with CSF β-amyloid and τ (Montine et al., 2001). Serial CSF F 2 isoprostanes, corrected for brain atrophy, may be useful markers of the efficacy of antioxidant interventions. We have not found plasma F 2 isoprostanes or urinary metabolites to be of diagnostic value in AD (Montine et al., 2002b), although others have found such levels to be informative (Pratico et al., 2002). Plasma measurements of F 2 isoprostanes are sensitive to systemic oxidative stress (Morrow et al., 1995) but do not appear to be altered by oxidative damage that is limited to the CNS (Montine et al., 2002b).

Clinical trials of phytochemicals in AD

**Ginkgo biloba**

A meta-analysis of *Ginkgo biloba* trials in dementia found only 4 out of 57 studies reviewed that met the criteria for meta-analysis and concluded that this agent was promising for dementia but that none of the studies, nor the meta-analysis, could confidently conclude that *Ginkgo biloba* is an effective treatment for AD (Oken et al., 1998). The meta-analysis, in vitro studies, and safety profile of this commonly used supplement have led to two publicly funded trials of *Ginkgo biloba* for the prevention of AD. The two studies differ in several respects and as a result are expected to provide complementary information.

The Dementia Prevention Study is being conducted in Portland, Oregon, with funding from the National Center for Complementary and Alternative Medicine. Healthy subjects aged 80 and older are randomized to...
Ginkgo biloba 240 mg per day (120 mg twice daily) versus placebo. Subjects have brain MRI scans with regional volume determination at baseline, followed by annual MRI scans. The primary end-point is progression to “mild cognitive impairment,” as progression from cognitive health to overt dementia does not occur in a timeframe that is feasible for clinical trials. In fact, this study is focused on the oldest old in order to detect a significant number of “converters” and will still have to follow subjects for 4 years in order to detect a prevention effect. Although this timeframe is an invitation to the type of drop-out that confounds analysis, only 14 out of 134 enrolled subjects have dropped out 3 years after enrollment, with 10 of those drop-outs due to death in this very old group.

The secondary end-point in this study is rate of brain volume loss, an outcome measure that will permit the distinction of a neuroprotectant effect from a symptomatic effect, in the case of a difference in clinical outcomes between Ginkgo biloba and placebo. The brain volume data may also be valuable in the event that the groups do not differ clinically, potentially yielding information on treatment effects that are below the detection threshold of clinical measurements. Plasma is also being collected from these subjects annually in order to determine if Ginkgo biloba treatment has an impact on plasma markers of systemic oxidative damage, including plasma F₂ isoprostanes and protein carbonyls.

The Ginkgo Evaluation of Memory (GEM) Study is a large, multicenter trial funded by the National Center for Complementary and Alternative Medicine, the National Institute on Aging, and the National Heart, Lung, and Blood Institute. The aims are to determine if Ginkgo biloba will slow the development of dementia, reduce rates of cognitive loss in aging, reduce vascular disease symptoms, and decrease total mortality. As in the Oregon study, the dose of Ginkgo biloba is 240 mg per day, and the duration of follow-up is extensive; 5 years in the GEM study. Participants are healthy subjects over the age of 75 and enrolled in the Cardiovascular Health Study in Pittsburgh; Baltimore; Durham, North Carolina; and Davis, California. The study is fully enrolled with 3000 subjects. Plasma is being collected and banked.

Curcumin
A pilot study of curcumin for the treatment of AD is being conducted at UCLA with funding from the Institute for the Study of Aging. Forty subjects with AD will be randomized to curcumin versus placebo. In addition to standard cognitive, functional, and behavioral outcome measures, subjects will have CSF collected for measurements of markers of inflammation and oxidative damage. This study is recruiting subjects at present.
Table 2. Clinical outcomes in a pilot study of Uncaria tomentosa for ADa.

<table>
<thead>
<tr>
<th>Gender (M/F)</th>
<th>Age at entry</th>
<th>Baseline MMSE</th>
<th>Δ MMSE 12 monthsb</th>
<th>Baseline ADAS</th>
<th>ΔADAS 12 monthsb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>13/7</td>
<td>72 ± 8</td>
<td>19.8 ± 5</td>
<td>−4.3 ± 5.2</td>
<td>23.5 ± 12</td>
</tr>
<tr>
<td>Placebo</td>
<td>13/7</td>
<td>70 ± 6</td>
<td>19.2 ± 5</td>
<td>−3.2 ± 3.8</td>
<td>25.2 ± 10</td>
</tr>
</tbody>
</table>

aMMSE, mini-mental state examination; ADAS, Alzheimer disease assessment scale. There were no significant differences in any measure at baseline or at 12-month follow-up.
bAt 12 months, 16 subjects remained in the active group; 20 in the placebo group.

Uncaria tomentosa

Based on in vitro data demonstrating inhibition of β-amyloid fibril formation, a pilot study of a proprietary extract of Uncaria tomentosa (PTI-00703) for the treatment of AD was conducted at the Oregon Aging and Alzheimer’s Center with funding from Rexall, Inc. and the Dana Foundation. Forty subjects with mild AD were randomized to PTI-00703 versus placebo at a dose of 350 mg three times daily. The treatment period was 1 year. Clinical outcome measures included the mini-mental state examination (MMSE) (Folstein, 1975) and the Alzheimer disease assessment scale (ADAS-cog) (Mohs & Cohen, 1988). Additional outcome measures included rates of brain atrophy, derived from brain MRI scans (Kaye et al., 1997) completed at baseline and at 12 months, and CSF levels of β-amyloid 1–42, τ, and F2 isoprostanes taken at baseline, 3 months, and 12 months.

All subjects completed the first 3 months of study. Four subjects dropped out after the third month due to one death, one adverse event leading to cessation of study drug, one case of inadvertent noncompliance, and one case of withdrawal of consent.

The study population at baseline consisted of 26 men and 14 women, with a mean age of 71 ± 7 years, mean MMSE = 19.5 ± 5; mean ADAS = 24 ± 10; mean ADL (Activities of Daily Living) = 31 ± 11; mean Instrumental Activities of Daily Living (IADL) = 6 ± 3. There were no significant differences between those randomized to active treatment and to placebo (Table 2).

All 40 subjects completed the first 3 months of the trial. At 3 months, a second CSF sample was collected for β-amyloid determination. β-amyloid levels were determined in 39 of 40 subjects at 3 months. The change in CSF β-amyloid level did not distinguish PTI-00703–treated subjects from placebo-treated subjects at 3 months. The active treatment group had a mean increase in CSF β-amyloid of 93 ± 123 pg/ml, whereas the placebo group had a mean increase in CSF β-amyloid of 78 ± 180 pg/ml.

All 20 subjects on placebo completed all 12 months of the study. Four subjects on active treatment dropped out between 3 and 12 months (one subject died, one subject stopped study drug after the onset of seizures, one subject withdrew consent, and one was discovered to be inadvertently noncompliant with study drug). None of the clinical measurements distinguished PTI-00703–treated subjects from placebo (Table 3).

All 36 subjects completing the study had another brain MRI at 12 months, with measurement of hippocampal volume, temporal lobe volume, total brain volume, and total ventricular volume, and annual rates of volume change in each of these regions was calculated. Change in brain volumes failed to distinguish PTI-00703–treated subjects from placebo-treated subjects (Table 3).

Thirty-three of the 36 subjects completing the study had CSF collected at 12 months, as three subjects declined the final lumbar puncture despite complying with all other study assessments. The change in CSF β-amyloid from baseline to 12 months did not distinguish PTI-00703–treated from placebo-treated subjects (Table 3).

To summarize, treatment with PTI-00703 at a dose of 350 mg p.o. three times a day for 1 year failed to slow the rate of clinical decline or the rate of brain atrophy in this sample of subjects with AD. Furthermore, no effect of PTI-00703 upon CSF β-amyloid was apparent. This study, however, generated abundant preliminary data for future clinical trials employing biological outcome measures.

Table 3. Biomarker outcomes in a pilot study of Uncaria tomentosa for ADa.

<table>
<thead>
<tr>
<th></th>
<th>Δ hippocampal volume</th>
<th>Δ temporal lobe volume</th>
<th>Δ total brain volume</th>
<th>Δ CSF β-amyloid</th>
<th>Δ CSF tau</th>
<th>Δ CSF F2 isoprostanes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>−0.04 ± 0.12</td>
<td>−3.4 ± 6</td>
<td>−16.6 ± 23</td>
<td>79 ± 109</td>
<td>−56 ± 335</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Placebo</td>
<td>−0.01 ± 0.12</td>
<td>−4.3 ± 4.8</td>
<td>−16.6 ± 18</td>
<td>90 ± 135</td>
<td>81 ± 207</td>
<td>9 ± 2</td>
</tr>
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</table>

aMRI measurements were completed for 16 treated and 20 placebo subjects and are expressed in cc per year. CSF measurements were completed for 15 treated and 18 placebo subjects and are expressed as pg/ml.
Considerations for future clinical trials of phytochemicals for AD

AD prevention versus treatment

Although effective prevention is probably more biologically feasible than effective treatment in the case of AD, demonstrating a treatment effect is logistically easier than demonstrating a prevention effect. The natural history of established AD is predictable enough to power clinical trials to be completed in the space of 6–12 months with a few hundred subjects. The onset of AD in healthy subjects, however, is so unpredictable and so gradual that prevention studies typically require very long follow-up, usually to a “pre-dementia” end-point rather than to dementia per sé, as exemplified by the ginkgo studies described above. Biomarkers of disease, including brain volumes, may serve as surrogate end-points in prevention studies, but this possibility has not yet been demonstrated. Additional biomarkers and better definitions of high-risk subjects for prevention studies may permit more definitive AD prevention studies in the future.

Choice of phytochemicals

The quality of the agent used in any clinical trial is a crucial factor, and in the case of complex extracts the issues are complicated many times over. The GEM study elected to use the Schwabe *Ginkgo biloba* extract, as this agent is the one used most widely in both basic and clinical research. The Oregon Dementia Prevention Study and the transgenic mice studies described above elected to use an extract from Thorne Pharmaceuticals (Sandpoint, IO, USA). This extract is prepared in standardized fashion, according to Good Manufacturing Procedures, with the goal of yielding 24% flavonoids and 6% terpenoids, similar to the Schwabe extract. For the sake of documenting the composition and assuring stability over time, samples were randomly analyzed by High Performance Liquid Chromatography (HPLC) fingerprinting by a contractor independent from the manufacturer. The material used in the *Uncaria tomentosa* study was a proprietary extract, which had been shown to solubilize β-amyloid fibrils in vitro.

Delivery of phytochemicals: Bioavailability and CNS penetration

Although it is theoretically possible for a therapeutic agent to exert beneficial effects on the brain indirectly from within the GI tract or within the plasma (e.g., via β-amyloid chelation), most agents for the treatment of neurologic disease must be delivered to the plasma and brain. The demonstration of bioavailability requires an assay for active ingredients that is suitable for use with plasma and/or CSF. Because the active ingredients of complex extracts are often not known, this sort of assay is sometimes impossible to achieve. If CNS effects of an orally administered candidate agent can be demonstrated, however, as in the memory improvement in the ginkgo-treated animals described above, then the direct demonstration of bioavailability and CNS penetration may be less crucial for designing and justifying a clinical trial.

Due to uncertainty regarding active ingredients, the clinical trials described with *Ginkgo biloba* are being conducted without a rigorous demonstration of bioavailability or CNS penetration of the agent, a reasonable approach given the abundant animal data showing CNS effects of orally administered *Ginkgo biloba* (Winter, 1991; Barkats et al., 1995). Similarly, the curcumin trial relies on animal studies showing a CNS effect of oral curcumin rather than a definitive demonstration of plasma and CSF levels of curcumin in human subjects (Lim et al., 2001). In the *Uncaria tomentosa* trial, however, the lack of bioavailability and CNS penetration data limits the ability to draw definitive conclusions regarding the negative outcome, which may have been due to failed absorption, failed CNS penetration, inability to solubilize β-amyloid fibrils in the brain parenchyma (as compared to the in vitro conditions), or inadequacy of dose.

Dosing and duration of therapy

Whether bioavailability and CNS penetration are demonstrated or inferred, the problem of appropriate dosing remains. Extrapolating from an animal dose to a human dose is not trivial. The method of “inter-species scaling” (Mahmood, 2002) may be used for predicting clearance rates and volume of distribution in human subjects in the case of single agents, but this approach is limited in the case of complex extracts, especially when active ingredients are unknown. An alternative approach may be to aim for a steady-state human plasma level of a measurable component of the extract, equivalent to the steady-state plasma level achieved in animal studies showing neurologic benefits.

None of the animal studies described above measured plasma levels of active ingredients or surrogate markers of the therapeutic agent, so the adequacy of dosing in clinical trials in these cases is being guided by other factors. The ginkgo studies are using a dose that has been used in previous human studies with favorable results and no significant toxicity (Oken et al., 1998). The curcumin trial is using a dosing information from previous colon cancer studies (Greenwald et al., 2002). The *Uncaria tomentosa* trial used a dose calculated from in vitro data, making gross assumptions about bioavailability, with the consequent limitations on interpretation of results as described above.
Measuring efficacy at the level of targeted mechanisms

Measuring efficacy is always a challenge in indolent diseases like AD, whether the goal is prevention of progression in established disease or prevention of disease in healthy subjects. An inability to detect a treatment effect may be traced to several levels of analysis, such as failure to influence the mechanism of interest, failure of a successfully modified mechanism to impact the disease, or simple insensitivity of clinical measurements. To take the example of the *Uncaria tomentosa* trial, the absence of a clinical benefit might be due to failure to solubilize β-amyloid or failure of this strategy to influence the disease course. Alternatively, effective β-amyloid solubilizing and effective neuroprotection might not be reflected in clinical measures. Because there was no effect of treatment on serial CSF β-amyloid level, we are inclined to conclude that the study failed on the basis of failure to solubilize β-amyloid, rather than failure of the general strategy, an important conclusion for the sake of designing future trials. Similarly, the inclusion of indices of inflammation and oxidative damage in the curcumin trial and inclusion of brain volumes in the ginkgo dementia prevention study will permit a more definitive interpretation of the results of these studies in progress.

Conclusions

Armed with data from a relatively new animal model, clinicians are launching a number of trials of polyphenolics and other phytochemicals for AD. The ability to measure the components of candidate agents in human body fluids is crucial to the issue of demonstrating adequate bioavailability and dosing in human studies, particularly for the interpretation of negative results. The use of other biomarkers of β-amyloid production, clearance, and toxicity, including inflammatory and oxidative damage, are also crucial to the conduct and interpretation of these clinical trials. The battery of useful biomarkers continues to expand, and the studies currently underway may permit additional validation of these measurements.

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