Effects of *Gingko biloba* supplementation in Alzheimer’s disease patients receiving cholinesterase inhibitors: Data from the ICTUS study

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\textbf{A R T I C L E   I N F O}

Article history:
Received 13 August 2013
Received in revised form 26 October 2013
Accepted 14 January 2014

Keywords:
Gingko biloba
Alzheimer’s disease
Cholinesterase inhibitors
Cognitive impairment

\textbf{A B S T R A C T}

*Gingko biloba* (Gb) is currently the most investigated and adopted herbal remedy for cognitive disorders and Alzheimer’s disease (AD). Nevertheless, its efficacy in the prevention and treatment of dementia still remains controversial. Specifically, the added effects of Gb in subjects already receiving “conventional” anti-dementia treatments have been to date very scarcely investigated. We evaluated whether the use of Gb is associated with additional cognitive and functional benefit in AD patients already in treatment with cholinesterase inhibitors (ChEIs).

Data are from mild to moderate AD patients under ChEI treatment recruited in the Impact of Cholinergic Treatment USE (ICTUS) study. Mixed model analyses were performed to measure six-monthly modifications in the Mini Mental State Examination (MMSE), the Alzheimer’s Disease Assessment Scale-Cognitive (ADAS-Cog) subscale score, and the Activities of Daily Living (ADL) scale over a follow-up of 1 year according to the additional Gb supplementation.

A total of 828 subjects were considered for the present analyses. Significantly different modifications at the MMSE score over the 12-month follow-up were reported between patients on combined therapy compared to those only taking ChEIs. On the contrary, the modification of the ADAS-Cog score between the two groups did not show statistically significant differences, although similar trends were noticed. No significant modifications of the two adopted outcome measures were observed at the mid-term 6-month evaluation. The modifications over time of the ADL score did not show statistically significant differences between the two groups of interest.

Our findings suggest that Gb may provide some added cognitive benefits in AD patients already under ChEIs treatment. The clinical meaningfulness of such effects remains to be confirmed and clarified.

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http://dx.doi.org/10.1016/j.phymed.2014.01.003

Please cite this article in press as: Canevelli, M., et al., Effects of *Gingko biloba* supplementation in Alzheimer’s disease patients receiving cholinesterase inhibitors: Data from the ICTUS study. Phytomedicine (2014). http://dx.doi.org/10.1016/j.phymed.2014.01.003
Introduction

In the last decades, several natural products have been tested for preventing the onset of dementia or delay its progression. Ginkgo biloba (Gb) is currently the most investigated and adopted herbal remedy for cognitive disorders and Alzheimer’s disease (AD) (Weinmann et al., 2010), and is listed as an anti-dementia drug in the Anatomical Therapeutic Chemical Classification system. Plausible mechanisms of its action against AD include antioxidant and antiapoptotic properties as well as potential inhibiting effects against caspase-3 activation and amyloid-β aggregation (Luo et al., 2002). Despite being widely used and tested, the efficacy of Gb in the prevention and treatment of dementia still remains controversial. Recently, two large randomized controlled trials (i.e. the Ginkgo Evaluation of Memory (GEM) (DeKosky et al., 2008) and the GuidAge (Vellas et al., 2012) studies) found no favorable effects of Gb in primary prevention of dementia and AD in older individuals. In contrast, several studies have suggested that Gb is more effective than placebo in improving cognition in cohorts of patients with dementia (that is in secondary prevention) (Weinmann et al., 2010; Ihl, 2012). The few studies comparing the efficacy of Gb versus cholinesterase inhibitors (ChEIs) in treating mild to moderate AD patients reported substantially comparable effects (Mazza et al., 2006; Yancheva et al., 2009; Schulz, 2003; Wettstein, 2000). However, it is noteworthy the almost complete lack of studies testing the added effects of Gb in subjects already receiving “conventional” anti-dementia treatments (e.g. ChEIs) (Weinmann et al., 2010; Yancheva et al., 2009).

In the present study, we hypothesize that Gb may provide additional cognitive and/or functional benefits in AD when combined to “first-line” treatments. The aim of these analyses is thus to evaluate the added effects of a Gb intervention in mild to moderate AD patients already in treatment with ChEIs. Therefore, we measured the longitudinal modifications (occurred over a one-year follow-up) of the Mini Mental State Examination (MMSE) (Folstein et al., 1975), of the Alzheimer’s Disease Assessment Scale–Cognitive (ADAS-Cog) subscale (Rosen et al., 1984), and of the Activities of Daily Living (ADL) scale (Katz et al., 1963) scores according to the additional Gb therapy in the Impact of Cholinergic Treatment Use (ICTUS) study.

Methods

Study design and participants

The ICTUS study has been previously described elsewhere (Reynish et al., 2007; Canevelli et al., 2013). Briefly, the ICTUS study is a prospective multicenter cohort study aimed at evaluating the clinical course, treatment outcomes, and socioeconomic impact of AD in Europe. It involved 29 participating centers from 12 European countries, all members of the European Alzheimer Disease Consortium (EADC), a network of clinical and research institutions specialized in the diagnosis and treatment of AD.

The following inclusion criteria were adopted in the ICTUS study: (1) diagnosis of probable AD made according to National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984); (2) MMSE (Folstein et al., 1975) score ranging from 10 to 26; (3) living in the community with a well-identified informal caregiver; (4) absence of known conditions reducing to less than 2 years the patient’s life expectancy; (5) ability to sign an informed consent. The study was approved by the Ethics Committee of the Toulouse University Hospital (coordinating center) and at individual centers by local or national ethical committees. All the study participants signed informed consent.

After the baseline assessment (between February 2003 and July 2005), participants were followed up for 2 years with mid-term re-evaluations every 6 months. At baseline and at each follow-up visit, a comprehensive clinical and neuropsychological assessment was performed. In particular, the scales and questionnaires that were administered to evaluate the neurological, functional, and social factors of participants were the following: Clinical Dementia Rating (Morris, 1993), MMSE (Folstein et al., 1975), ADAS-Cog (Rosen et al., 1984), Zarit Burden Interview (ZBI) (Zarit et al., 1980), Neuropsychiatric Inventory (NPI) (Cummings et al., 1994), ADL (Katz et al., 1963), and Instrumental Activities of Daily Living (IADL) scale (Lawton and Brody, 1969). Moreover, at every visit, concomitant pharmacological treatments were recorded.

For the present analyses, ICTUS participants were divided in two groups: (1) AD patients receiving only ChEIs (i.e. donepezil, rivastigmine, and galantamine) at the baseline and 12-month follow-up visits; and (2) AD patients receiving ChEIs and an additional Gb supplementation at the baseline and 12-month follow-up visits. All the patients on combined therapy were receiving the Gb extract EGb761, at a daily dosage of 120 mg in most of cases (56%). Participants not receiving ChEIs (e.g. in treatment with memantine) were not considered as well as those changing group of treatment during the considered follow-up. Such analytical approach allowed us to mirror (although with the obvious limitations of a cohort study) the design of a clinical trial (i.e., two groups exposed to different treatments for the same length of time).

The present analyses were restricted to the first 12 months in order to avoid a potential selection bias. In fact, it is likely that subjects taking Gb for a longer time are those who have benefited more from the therapy. Thus, extending the period of observation may have led to the selection of participants mainly presenting a positive response to treatment.

Cognitive function tests

Modifications of the MMSE and ADAS-Cog scores after 12 months of follow-up were considered as cognitive outcome variables of interest. The MMSE (Folstein et al., 1975) includes 30 items focused at measuring different cognitive aspects (orientation, registration, attention, recall, and language). The total score ranges from 0 to 30 with higher scores indicating better cognitive performance.

The ADAS-Cog (Rosen et al., 1984) represents the most widely adopted cognitive outcome measure in AD trials. It includes eleven items assessing different cognitive domains (memory, language, and praxis). The total ADAS-Cog score ranges from 0 to 70, with higher scores indicating greater cognitive impairment.

Functional ability tests

Modifications of the ADL scale scores after 12 months of follow-up were considered as functional outcome variable of interest. The IADL scale was not considered for the present analyses because poorly reliable as functional measure in elderly men (Fusco et al., 2012). The ADL scale (Katz et al., 1963) is a carer-applied questionnaire ranking adequacy of performance in the 6 functions of bathing, dressing, toileting, transferring, continence, and feeding. Patients score 1 for independence in each of the 6 functions. Higher scores indicate greater functional independence.

Other variables

The following potential confounding factors were considered in the present analyses: age, gender, educational level, income, and their interaction with time.
Table 1
Baseline characteristics of the cohort. Values are expressed as % or means ± SDs.

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 828)</th>
<th>ChEIs (n = 799)</th>
<th>ChEIs + Gb (n = 29)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>75.8 ± 7.8</td>
<td>75.8 ± 7.8</td>
<td>76.2 ± 6.8</td>
<td>0.74</td>
</tr>
<tr>
<td>Gender (women)</td>
<td>64.7</td>
<td>64.8</td>
<td>62.1</td>
<td>0.76</td>
</tr>
<tr>
<td>Education [years]</td>
<td>7.9 ± 4.6</td>
<td>7.8 ± 4.6</td>
<td>10.0 ± 4.9</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>25.3 ± 4.1</td>
<td>25.2 ± 4.0</td>
<td>26.0 ± 5.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Hypertension</td>
<td>37.7</td>
<td>37.7</td>
<td>37.9</td>
<td>0.98</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12.2</td>
<td>12.3</td>
<td>10.3</td>
<td>0.99</td>
</tr>
<tr>
<td>Ischemic heart diseases</td>
<td>12.6</td>
<td>12.5</td>
<td>13.8</td>
<td>0.78</td>
</tr>
<tr>
<td>Depression</td>
<td>24.3</td>
<td>24.0</td>
<td>31.0</td>
<td>0.39</td>
</tr>
<tr>
<td>Stroke</td>
<td>7.5</td>
<td>7.5</td>
<td>6.9</td>
<td>0.99</td>
</tr>
<tr>
<td>Falls</td>
<td>16.3</td>
<td>16.8</td>
<td>3.4</td>
<td>0.07</td>
</tr>
<tr>
<td>NPI total score</td>
<td>11.9 ± 12.8</td>
<td>12.1 ± 13.0</td>
<td>8.2 ± 6.4</td>
<td>0.19</td>
</tr>
<tr>
<td>ZBI</td>
<td>20.7 ± 14.7</td>
<td>20.8 ± 14.7</td>
<td>17.8 ± 13.8</td>
<td>0.31</td>
</tr>
<tr>
<td>MMSE</td>
<td>20.5 ± 3.9</td>
<td>20.5 ± 3.9</td>
<td>21.2 ± 3.5</td>
<td>0.36</td>
</tr>
<tr>
<td>ADAS-Cog</td>
<td>20.4 ± 8.9</td>
<td>20.6 ± 8.9</td>
<td>15.8 ± 7.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADL</td>
<td>5.5 ± 0.8</td>
<td>5.5 ± 0.9</td>
<td>5.8 ± 0.4</td>
<td>0.06</td>
</tr>
</tbody>
</table>

ADAS-Cog: Alzheimer Disease Assessment Scale-Cognitive subscale; ADL: Activities of Daily Living; ChEIs: cholinesterase inhibitors; Gb: Ginkgo biloba; MMSE: Mini Mental State Examination; NPI: Neuropsychiatric Inventory; ZBI: Zarit Burden Interview.

Statistical analysis

In order to compare the baseline characteristics between the 2 treatment groups, we used χ² or Fisher’s exact (for expected values <5) test for categorical variables, Student test for quantitative variables with Gaussian (normal) distributions, and non-parametric tests (Kruskal–Wallis test) for quantitative variables without normal distributions. In the absence of a normal distribution variables were transformed and tested on square root (for age, NPI and ZBI) or logarithmic values (BMI and ADAS-Cog) in order to obtain normal distributions.

In order to compare changes over time for MMSE, ADAS-Cog, and ADL scores between the two patient groups: those receiving ChEIs alone and those treated by the combination ChEIs and Gb, linear mixed effect models with random intercept and random slope were used to take into account the heterogeneity of baseline scores and individual slopes over time. The term time² was significant, showing that the MMSE, ADAS-Cog, and ADL slope are quadratic.

Two models were used either for MMSE, ADAS-Cog, and ADL. The first one was an unadjusted model. The second model was adjusted for: age, gender, education, income, and their interaction with time. In addition for the second model we used a linear mixed model with 3 levels in order to take into account the two levels of correlation: between repeated observations of individual patients and between patients within country. The statistical package SAS 9.3 software (SAS Institute Inc., Cary, NC) was used for the present analyses.

Results

Descriptive characteristics of the study sample at the baseline assessment are shown in Table 1. A total of 828 patients (women 64.7%) were considered for the present analyses. The sample population had a mean age of 75.8 (SD 7.8) years. MMSE and ADAS-Cog scores at baseline were 20.5 (SD 3.9) and 20.4 (SD 8.9), respectively, indicating a moderate cognitive decline. ADL score was 5.5 (SD 0.8), indicating a minimal impairment of functional abilities. At the baseline and 12-month visits, 799 participants (96.5%) were on ChEIs alone treatment, and 29 (3.5%) were on a combined therapy of ChEIs and Gb. At the baseline visit, donepezil was the most assumed ChEI (55%) followed by galantamine (27%) and rivastigmine (18%).

The two groups were comparable for age, gender, and comorbidities. Significant differences between them were reported for education (p = 0.01) and ADAS-Cog scores at baseline (p < 0.01).

In particular, participants on combined treatment were found to be more educated and less cognitively impaired.

The different modifications of MMSE and ADAS-Cog scores in the two groups of interest are presented in Table 2. Significant different modifications at the MMSE score over the 12-month follow-up were reported between patients on combined therapy compared to those only taking ChEIs (+1.86, SE 0.67; p = 0.006). These results were confirmed in the multivariate model after adjusting for potential confounders (+1.91, SE 0.67; p = 0.005). On the contrary, the modification of the ADAS-Cog score between the two groups did not show statistically significant differences, although similar trends were noticed. Similarly, no significant modifications of the two adopted outcome measures were observed in secondary analyses exploring cognitive function modifications at the mid-term 6-month assessment.

The modifications over time of the ADL score did not show statistically significant differences between the two groups of interest (Table 3).

Discussion

In the present study, we explored modifications of cognitive and functional performance over one year of follow-up in a large cohort of mild to moderate AD patients treated with ChEIs, according to the additional Gb use. A significant difference of the MMSE modifications was reported between participants using the combined therapy compared to those only taking ChEI after one year of follow-up. A similar, but not statistically significant trend was found for the ADAS-Cog modifications. Conversely, no differences in terms of functional ability (i.e. ADL score modifications) were observed over time between the two groups of interest.

The clinical meaningfulness of our findings remains to be clarified. The cognitive benefit observed among patients on combined therapy (as measured by an increase of the MMSE score) was found to be statistically significant only at the 12-month, but not at the 6-month assessment. The partially positive results obtained for the Gb at the end of the 12-month follow-up might be due to our study design (analyses from a cohort study, and not from a randomized controlled trial). The cohort study design does not allow us to surely ascertain the period of exposure and adherence of participants to the treatments of interest (i.e. subjects may shift groups at any time during the period of observation or irregularly assume the treatments). For this reason, we performed our analyses selecting participants taking the same treatment during the first 12-month follow-up of ICTUS. However, we cannot not exclude that
participants perceiving an amelioration of their health status from the Gb treatment were indeed those more likely to be included in the group on combined therapy. On the other hand, those feeling the combined therapy as unworthy probably quit the Gb treatment after a short time of trial (also considering the treatment costs), thus were likely to be excluded from the present study analyses. As a consequence, an overestimation of our findings, especially for the 12-month assessment, needs to be considered. Basing on the same considerations, we also limited our analyses to 12 months, not considering the cognitive and functional modifications occurred in the second year of follow-up of the ICTUS cohort. In fact, it is likely that patients experiencing the greatest benefit from the added Gb therapy would have more probably completed the observation period compared to participants reporting minor efficacy. This may have further increased the above-described selection bias.

Based on available evidence, Gb may potentially represent an “interesting” add-on therapy in demented subjects already receiving “conventional” pharmacological treatments. In fact, it is well tolerated (Weinmann et al., 2010) and may provide additional benefits by targeting different pathophysiological mechanisms. To our knowledge, only one study had previously investigated the cognitive efficacy of a combined ChEIs+Gb treatment in AD (Yancheva et al., 2009). In this study, 96 AD outpatients were randomly assigned to Gb (240 mg/day), donepezil (initially 5 mg/day, then 10 mg/day after 4 weeks), or to the combined treatment (same doses). After 22 weeks, no significant differences concerning cognitive, behavioral, and functional outcomes were noticed between the three treatment groups. Interestingly, compared to donepezil monotherapy, the adverse event rate was lower under Gb treatment and even under the combination treatment. Nevertheless, the small sample size did not allow any definitive conclusion. Also, the use of cognitive measures (i.e. Syndrom Kurz Test, Clock-Drawing Test, and Verbal Fluency Test) different from those available in the ICTUS study does not allow a direct comparison with our findings.

Our study has several strengths. The analyses were performed in a large sample of AD patients, recruited at numerous dementia clinics across several European countries. The modification of cognitive performance was assessed through two widely used outcome measures (i.e. the MMSE, and ADAS-Cog) aiming at reducing observation bias. Moreover, the study design with semi-annual clinical assessments provided a detailed monitoring of cognitive changes. Nevertheless, some issues should be discussed because potentially influencing our results. First, the observational design did not allow us to conclude in terms of causality. In fact, the two groups were significantly different with regard to education and ADAS-Cog scores at baseline, two well-established factors associated with the course of the disease, the performance at cognitive testing, and the response to treatments. Thus, it may be hypothesized that patients on combined ChEIs+Gb therapy may have presented a more relevant cognitive benefit because more educated and less cognitively impaired. So, even if these factors were

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Different evolutions of MMSE and ADAS-Cog scores according to the two groups of interest (baseline–follow-up). Values are expressed as means ± SEs (Standard Errors). Results from linear mixed models.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMSE 6 months</strong></td>
<td><strong>Mean differences (follow-up – baseline)</strong></td>
</tr>
<tr>
<td>ChEIs</td>
<td>-0.41 ± 0.10</td>
</tr>
<tr>
<td>ChEIs + Gb</td>
<td>+0.51 ± 0.54</td>
</tr>
<tr>
<td><strong>MMSE 12 months</strong></td>
<td><strong>Mean differences (follow-up – baseline)</strong></td>
</tr>
<tr>
<td>ChEIs</td>
<td>-1.44 ± 0.12</td>
</tr>
<tr>
<td>ChEIs + Gb</td>
<td>+0.42 ± 0.66</td>
</tr>
<tr>
<td><strong>ADAS-Cog 6 months</strong></td>
<td><strong>Mean differences (follow-up – baseline)</strong></td>
</tr>
<tr>
<td>ChEIs</td>
<td>+1.25 ± 0.20</td>
</tr>
<tr>
<td>ChEIs + Gb</td>
<td>+0.45 ± 1.09</td>
</tr>
<tr>
<td><strong>ADAS-Cog 12 months</strong></td>
<td><strong>Mean differences (follow-up – baseline)</strong></td>
</tr>
<tr>
<td>ChEIs</td>
<td>+3.41 ± 0.25</td>
</tr>
<tr>
<td>ChEIs + Gb</td>
<td>+2.00 ± 1.33</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, education, income, and their interaction with time.

Negative differences at the ADAS-Cog indicate improvement of cognitive function. Positive differences at the MMSE indicate improvement of cognitive function.

ADAS-Cog: Alzheimer Disease Assessment Scale-Cognitive subscale; ChEIs: cholinesterase inhibitors; Gb: Ginkgo biloba; MMSE: Mini Mental State Examination.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Different evolutions of ADL scores according to the two groups of interest (baseline–follow-up). Values are expressed as means ± SEs (Standard Errors). Results from linear mixed models.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADL 6 months</strong></td>
<td><strong>Mean differences (follow-up – baseline)</strong></td>
</tr>
<tr>
<td>ChEIs</td>
<td>-0.16 ± 0.01</td>
</tr>
<tr>
<td>ChEIs + Gb</td>
<td>-0.19 ± 0.08</td>
</tr>
<tr>
<td><strong>ADL 12 months</strong></td>
<td><strong>Mean differences (follow-up – baseline)</strong></td>
</tr>
<tr>
<td>ChEIs</td>
<td>-0.32 ± 0.03</td>
</tr>
<tr>
<td>ChEIs + Gb</td>
<td>-0.38 ± 0.16</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, education, income, and their interaction with time.

Negative differences at the ADL indicate decline of functional abilities.

ADL: Activities of Daily Living; ChEIs: cholinesterase inhibitors; Gb: Ginkgo biloba.
properly taken into account in the adjusted models, the consequent bias might have not been completely erased. Moreover, despite considering potential confounders, third factors may have affected or may differently explain our findings. For example, the treatment doses were not stable and uniform during the study, healthier patients may have easier access to the Gb treatment, and the concomitant use of other psychoactive drugs may have interacted with the tested pharmacological interventions. The study design and available data also do not allow appreciating and adequately taking into account the possible exposure to the Gb before the ICTUS baseline visit. For example, it is possible a residual effect of previously stopped Gb treatment in the ChEi group as well as an overestimation of benefits in participants having been taken the combined therapy for several years before. Finally, our analyses might have been affected by the low number of participants taking Gb during the period of interest.

In conclusion, our findings suggest that the Gb might provide some added cognitive benefits in AD patients already under ChEIs treatment. However, the clinical relevance of such effects remains to be confirmed and clarified in future ad hoc designed trials.

Conflict of interest

No disclosures to report.

Acknowledgements

The ICTUS study was partially supported by a grant from the European Commission within the 5th framework program (QLK6-CT-2002-02645) and partially from an unrestricted equal grant from each of Eisai, Jansen, Lundbeck, and Novartis pharmaceutical companies. The pharmaceutical companies had no role in study design, data collection, data analysis, data interpretation. Promotion of the ICTUS study was supported by the University Hospital Centre of Toulouse. The data sharing activity was supported by the “Association Monegasque pour la recherche sur la maladie d’Alzheimer” (AMPA) and the UMR 1027 Unit INSERM–University of Toulouse III.

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