An MRI substudy of a donepezil clinical trial in mild cognitive impairment

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Abstract

A magnetic resonance imaging (MRI) study was conducted as part of an intervention study in subjects with amnestic mild cognitive impairment (aMCI) to assess donepezil’s treatment effect on brain atrophy. Adults with aMCI were randomly assigned to double-blind treatment with 10 mg/day donepezil hydrochloride or placebo for 48 weeks. Brain MRI scans were acquired at baseline and endpoint. The primary outcome measure was annualized percentage change (APC) in hippocampal volume; the main secondary outcome measure was APC in whole brain volumes. An analysis of variance (ANOVA) model including terms for treatment, site, and age was used to compare the treatment groups. APCs for hippocampal volumes were not significantly different between treatment groups. There were significant differences favoring the donepezil group for total (\(p < 0.001\)), ventricular region (\(p < 0.0002\)), and cortical region (\(p < 0.003\)) whole brain volumes. Although the primary MRI outcome measure was negative, the main secondary MRI outcome measure showed a positive result. These findings suggest a treatment effect of donepezil on brain atrophy in aMCI.

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1. Introduction

The concept of mild cognitive impairment (MCI) describes a heterogeneous clinical condition with several subtypes and multiple etiologies (Petersen et al., 2001). The amnestic subtype (aMCI) is defined as a significant decline in memory with either slight or no impairment in activities of daily living (Petersen et al., 2001). As many as 80% of patients with aMCI have been reported to progress to Alzheimer’s disease (AD) within 6 years of diagnosis (Petersen et al., 2001), which is consistent with the finding that aMCI is often of a degenerative etiology (Morris et al., 2001). Together these data suggest that aMCI may represent the prodromal state of AD (Morris et al., 2001). As a result, cholinesterase inhibitors (ChEIs), the mainstay of treatment for AD, have been evaluated for the treatment of MCI.

Donepezil, an acetylcholinesterase inhibitor, has been shown to delay progression to AD over a period of 1 year, though the rate of progression to AD after 3 years was not lower among patients treated with donepezil (Petersen et al., 2005). Galantamine and donepezil have shown benefit in subjects with MCI on secondary outcome measures (Salloway et al., 2004; Winblad et al., 2008). Nonetheless, no agent tested in a randomized clinical trial in MCI has met its primary efficacy objectives, all of which have been defined by clinical rating instruments (Doody et al., 2009; Feldman et al., 2007; Petersen et al., 2001; Salloway et al., 2004;
Thal et al., 2005; Winblad et al., 2008). Therefore, an objective marker of disease progression in MCI has been sought to supplement the clinical rating scales and cognitive tests (Jelic et al., 2006).

Identifying how donepezil might slow neurodegenerative progression in aMCI in a manner that could be detected by structural magnetic resonance imaging (MRI) is complicated by the fact that the mechanisms responsible for neuronal loss in AD are largely unknown. However, evidence from clinical trials of donepezil in the treatment of AD provides support for the possibility that, compared with placebo, donepezil slows neurodegenerative progression in treated patients with AD (Hashimoto et al., 2005; Krishnan et al., 2003). These prospective, placebo-controlled studies demonstrated significantly reduced hippocampal brain atrophy in the donepezil-treated group compared with the control group. In addition, evidence from animal studies suggests that donepezil at clinically relevant concentrations can attenuate amyloid (Aβ25,35)-induced toxicity (Mennier et al., 2006; Svensson and Nordberg, 1998), which may slow the neurodegenerative process and thus stabilize brain atrophy. Therefore, if MCI is an early form of AD in a substantial proportion of patients, these preclinical and clinical data indicate that rates of hippocampal brain atrophy may be reduced with donepezil treatment in comparison with rates in a placebo-treated control group.

Serial MRI, which has been utilized extensively in studies of MCI, has shown significant differences between MCI patients and controls in rates of hippocampal and whole brain volume loss (Fox et al., 1996, 2000; Jack et al., 2004, 2008). In particular, higher rates of hippocampal atrophy in MCI were associated with generally higher cognitive decline, suggestive of accumulative AD structural changes (van de Pol et al., 2007). Moreover, several MRI studies demonstrated an association between increased rates of brain atrophy, including shrinkage of the hippocampus, and higher rates of clinical progression from MCI to AD (Misra et al., 2009; Spulber et al., 2008). Serial MRI scans (1–5 years apart) in 160 adults have shown that ventricular expansion and atrophy rates of the whole brain, hippocampus, and entorhinal cortex (ERC) were greater in cognitively normal subjects who progressed to MCI or AD than among those who remained stable, and greater among MCI subjects who converted to AD than among those who did not (Jack et al., 2004). In 131 subjects who had serial MRI scans during the vitamin E and donepezil study (Petersen et al., 2005), significant correlations between cognitive test performance and regional and whole brain atrophy rates were demonstrated (Jack et al., 2008), indicating that the neuronal correlate of clinical symptoms in MCI is progressive brain atrophy.

These data suggest that measurements of brain atrophy progression are associated with clinical symptoms of cognitive decline, may distinguish between MCI and normal aging, and may be used to evaluate the effects of therapeutic interventions in MCI. Therefore, an MRI substudy was included as part of an intervention study of donepezil in patients with aMCI (NCT00293176) (Doody et al., 2009). The primary hypothesis was that donepezil treatment would slow the rate of brain atrophy. A secondary hypothesis was that the rate of brain atrophy would correlate with clinical measures of cognitive decline.

2. Methods

2.1. Patient population

Participants comprised a subset from the primary clinical trial of donepezil for the treatment of aMCI (Clinicaltrials.gov NCT00293176). The details of the parent study have been previously described (Doody et al., 2009). Briefly, subjects (45–90 years of age) met criteria for aMCI (Petersen et al., 2001), with a recent (<1 year) brain scan showing no evidence of focal lesions. At screening evaluation, participants had a global Clinical Dementia Rating (CDR) score of 0.5, memory box score between 0.5 and 1.0 (Berg, 1988), Mini-Mental Status Examination (MMSE) score between 24 and 28 inclusive (Folstein et al., 1975), and Rosen-modified Hachinski ischemia scale score ≤4. Exclusionary criteria included current diagnosis of any major neurological, psychiatric, or substance use disorder; uncontrolled diabetes or hypertension; a sleep disorder that could affect cognitive performance; or treatment with a ChEI or memantine for >1 month or within 3 months of screening.

2.2. Study design

Subjects participated in a 3-week single-blind, placebo run-in period followed by a 48-week double-blind period during which they were randomly assigned to treatment with 10 mg/day donepezil hydrochloride or placebo (Fig. 1). The modified Alzheimer Disease Assessment Scale-cognitive subscale (ADAS-cog) (Doody et al., 2009; Rozzini et al., 2008) was administered at each visit except week 3, the CDR sum of boxes (SB) (O’Bryant et al., 2008) at each visit. The study protocol was approved by the institutional review board of each site, and informed consent was obtained from all subjects. Brain MRI scans were acquired at 36 qualified investigative sites at baseline and after 50 weeks of treatment, or after at least 6 months in subjects who terminated the study prematurely.

2.3. MRI methodology

The sponsor mandated CCBR-Synarc (San Francisco, California), a provider of central radiology services, to oversee the image acquisition, quality control, and off-site independent blinded central review of images performed at the Center for Imaging of Neurodegenerative Diseases. Procedures were put in place to ensure the standardization and quality of MRI acquisition and image assessment.

For each scan, fast scout scans were initially obtained to determine head position in the magnet, followed by coronal
3-dimensional T1-weighted images performed at $1 \times 1.5$-mm$^2$ resolution and angulated perpendicular to the long axis of the hippocampus. An axial T2-weighted fast spin echo sequence was obtained to determine intracranial volume (ICV) and to conduct clinical readings at the clinical site. An axial fluid-attenuated inversion recovery (FLAIR) sequence was collected to assess white matter lesions and lacunar infarcts at the clinical site.

MRI scans collected for the study were sent to Synarc for quality control and archiving, and then transferred to the lead author and his group for evaluation. Readers were blinded to treatment assignment, chronological order of images, and relevant clinical information. The main criteria for judging MRI quality included appearance of gray/white matter contrast, severity of image artifacts (e.g., ringing, ghosting, and head movements), and the ability to co-register each subject’s serial MRI scans. In the few cases that had discrepant readings, the lead author (NS) made the final decision for pass or fail.

Quantitative volumes of the ERC, hippocampus, and whole brain atrophy were obtained from the T1-weighted MRI data using established and previously published procedures. The ERC was traced manually (Du et al., 2001) following an established protocol (Insausti et al., 1998) (figs e–1A). Tracing of the left and right hippocampus was performed using a semiautomated brain mapping method based on a high-dimensional fluid transformation algorithm (figs e–1B) (Christensen et al., 1997). A commercially available version of the algorithm was used (Medtronic Surgical Navigation Technologies, Louisville, Colorado).

Whole brain atrophy was measured using the boundary shift integral (BSI) (Ezekiel et al., 2004) generally following established procedures (figs e–1C) (Freeborough and Fox, 1997). Measurements of whole brain atrophy were further classified into atrophy of periventricular regions and cortical regions based on tissue classifications using image segmentation software (Tanabe et al., 1997). The final markings of ERC, hippocampus, and BSI were checked visually by 2 experienced readers independently and either accepted or rejected by consensus.

### 2.4. Statistical analysis

Subjects included in the MRI substudy intent-to-treat (ITT) population were those who were randomized, received at least 1 dose of double-blind study medication, had baseline and at least 1 postbaseline assessment of at least 1 efficacy variable, and had a baseline and follow-up MRI scan. The MRI variables analyzed were rates of hippocampal, ERC, and whole brain volume change; hippocampal volume change was the primary outcome measure. Intracranial volume (ICV) was used as the primary outcome measure. Intracranial volume (ICV) was used as the numerator to derive head size-adjusted volumes.

Rate measurements were expressed as annualized percentage change (APC) from baseline volumes, according to:

$$\left(\frac{V_{ij} - V_{i0}}{V_{i0}}\right) \times \frac{365}{\Delta t_{ij}} \times 100$$

Here, $V_{ij}$ and $V_{i0}$ are the volumes of $i$-th subject measured at study baseline ($t = 0$) and end date ($t = j$), respectively; and $\Delta t_{ij}$ is the scan interval of the $i$-th subject. Rate measurements were further classified by apolipoprotein E (APOE) genotype (APOE e4 allele carriers versus noncarriers).

An analysis of variance (ANOVA) model with terms for treatment, site, age, and baseline MMSE category ($\leq 28$, $\geq 29$) was used to compare the treatment groups, as well as their corresponding APOE-defined subgroups, for each of these variables. Differences in least squares means, 95% confidence interval, and $p$ values for each variable were determined. The ANOVA tests of a treatment effect on brain volume changes were supplemented post hoc by linear
mixed effects analyses, in which the response variable (e.g., hippocampal volume) was regressed against an explanatory variable (e.g., time) as fixed effect, and variation in baseline volumes was included as random effect to account for a potential bias from smaller volumes.

To determine whether there was a relationship between cognitive measures and brain volumes, correlations between MRI assessments and clinical assessments (modifed ADAS-cog and CDR-SB) at baseline and at endpoint, as well as for annualized percentage change (APC), as defined above, were also investigated. Spearman’s coefficients were used to determine the correlation between these MRI and clinical variables, and p values (null hypothesis: \( \rho = 0 \)) were determined.

A post hoc analysis of the APC for hippocampal and ERC volumes was performed, restricted to those scan pairs that qualified for whole brain analysis, based on the criteria set forth for BSI quality control, because high variability and artifacts compatible with drifts in gradient calibration (which can mimic brain atrophy) were observed in the ITT dataset. The rationale for this analysis was to reduce these variations and potential systematic errors in the MRI data. Selection of these scan pairs was done completely blinded to subjects’ treatment status and baseline demographic and clinical characteristics.

3. Results

3.1. Subjects

Of the 2037 subjects screened, 821 subjects (donepezil, \( n = 409 \); placebo, \( n = 412 \)), were recruited from 74 sites and randomized; and 788 subjects were included in the ITT population (donepezil, \( n = 397 \); placebo, \( n = 391 \)) (Fig. 1). A total of 392 subjects participated in the MRI substudy, of whom 234 were included in the ITT population (donepezil, \( n = 109 \); placebo, \( n = 125 \)). APOE data were available for 74 patients (67.9%) in the donepezil group and 92 (73.6%) in the placebo group for these patients, 33 (44.6%) in the donepezil group and 43 (46.7%) in the placebo group were APOE e4 carriers. Demographic and baseline illness characteristics were similar for the MRI study subpopulation and the MCI total study population (Table 1).

3.2. MRI data

The mean interval between baseline and endpoint scans was 356 days (range, 205–479) for the donepezil group and 361 days (range, 252–424) for the placebo group. The number of scan pairs that passed the protocol-specified quality assessments was: ERC, 182 of 212; hippocampus, 215 of 234; and BSI, 164 of 234. Baseline volumes are shown in Table 2; differences between the placebo and treatment groups were not significant.

3.3. Hippocampal volume change

For the protocol-defined sample of scans, the APCs for total, left, and right hippocampal volumes were not significantly different between treatment groups (Table 3). There were also no significant treatment group differences when stratified by ApoE status (Table e–1).

For the post hoc sample of scans restricted to those that passed the BSI quality check, the between-group difference of the APC for right hippocampal volume was significant (\( p = 0.044 \)) and the between-group difference of the APC for
Data are given as mean ± standard error of the mean. All differences between placebo and donepezil were nonsignificant.

Table 3
Baseline volumetric measures (cubic centimeters)

<table>
<thead>
<tr>
<th>Brain measure</th>
<th>Placebo</th>
<th>n</th>
<th>Donepezil</th>
<th>n</th>
<th>Placebo</th>
<th>n</th>
<th>Donepezil</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hippocampal</td>
<td>4.044 ± 0.849</td>
<td>125</td>
<td>3.938 ± 0.757</td>
<td>105</td>
<td>4.113 ± 0.789</td>
<td>90</td>
<td>4.036 ± 0.832</td>
<td>72</td>
</tr>
<tr>
<td>Left hippocampal</td>
<td>1.991 ± 0.438</td>
<td>125</td>
<td>1.947 ± 0.375</td>
<td>105</td>
<td>2.053 ± 0.450</td>
<td>125</td>
<td>1.992 ± 0.410</td>
<td>105</td>
</tr>
<tr>
<td>Right hippocampal</td>
<td>2.053 ± 0.450</td>
<td>125</td>
<td>1.992 ± 0.410</td>
<td>105</td>
<td>4.113 ± 0.789</td>
<td>90</td>
<td>4.036 ± 0.832</td>
<td>72</td>
</tr>
<tr>
<td>Total entorhinal cortex (ERC)</td>
<td>1.076 ± 0.461</td>
<td>112</td>
<td>1.125 ± 0.440</td>
<td>100</td>
<td>1.118 ± 0.484</td>
<td>80</td>
<td>1.085 ± 0.358</td>
<td>70</td>
</tr>
<tr>
<td>Left ERC</td>
<td>0.531 ± 0.224</td>
<td>112</td>
<td>0.571 ± 0.242</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ERC</td>
<td>0.545 ± 0.265</td>
<td>112</td>
<td>0.555 ± 0.230</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total whole brain</td>
<td>1199 ± 127</td>
<td>74</td>
<td>1193 ± 135</td>
<td>74</td>
<td>35.00 ± 21.83</td>
<td>74</td>
<td>43.79 ± 29.93</td>
<td>74</td>
</tr>
<tr>
<td>Ventricular region</td>
<td>1164 ± 122</td>
<td>74</td>
<td>1149 ± 129</td>
<td>74</td>
<td>0.849 ± 1.125</td>
<td>105</td>
<td>0.818 ± 1.193</td>
<td>105</td>
</tr>
<tr>
<td>Cortical region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are given as mean ± standard error of the mean. All differences between placebo and donepezil were nonsignificant.

3.4. ERC volume change

For the protocol-defined and the post hoc sample of scans, the APCs for total, left, and right ERC volumes were not significantly different between treatment groups (Table 3).

There were also no significant treatment group differences when stratified for ApoE status (Table e–1). The linear mixed effects analyses were supportive of the original ANOVA (Table e–2).

3.5. Whole brain volume change

A significant difference was noted between treatment groups in the APC of total whole brain volume (p = 0.001), ventricular region whole brain volume atrophy (p = 0.0002), and cortical region whole brain volume (p = 0.003) (Table 3). For each of these measures, the donepezil group exhibited a slower rate of atrophy than the placebo group (Fig. 1). To exclude the possibility that slight differences in MRI scan intervals between the groups resulted in bias in rate measurements, we added variations in scan intervals to the model and obtained virtually identical results.

3.6. Correlational analysis

Most cross-sectional MRI measures correlated significantly with the ADAS-cog scores (Table 4). Specifically, the correlations between ADAS-cog scores and hippocampal, ERC, and total, cortical and ventricular whole brain measures were significant at baseline and endpoint (except for total whole brain volume at baseline). Fewer significant correlations were noted between CDR-SB scores and brain volume measures at baseline or endpoint (6 of 18, compared with 17 of 18 for ADAS-cog; Table 4).

The APC for the whole brain volumes correlated significantly with the APC for ADAS-cog scores, but not for CDR-SB scores. In contrast, the only significant correlations for the regional brain volume changes were between the APCs for total and right ERC volumes and the APC of CDR-SB, and between the APC for right hippocampal volume and the APC of ADAS-cog. These results were not appreciably altered in the post hoc sample of scans restricted to those that passed the BSI quality check (4 cor-
relations that had been significant for hippocampal measures were no longer significant).

4. Discussion

This study did not demonstrate a treatment effect of donepezil on hippocampal volume change over 1 year, the primary outcome measure, but did show a potential treatment effect of pharmacotherapy on other brain volume changes. Specifically, donepezil 10 mg/day was associated with slowed progression of whole brain atrophy over the course of 1 year. In this study, the difference between the donepezil and placebo groups on the primary outcome measure, APC in hippocampal volume, was not significant. However, the donepezil group did differ significantly from placebo on the secondary outcome measure, APC in whole brain volumes. This study is therefore the first clinical trial to demonstrate a treatment effect of donepezil on brain volume changes in aMCI. Moreover, the statistical significance of this effect was roughly an order of magnitude greater than that of the change in ADAS-cog ($p < 0.001$ versus $p = 0.01$) (Doody et al., 2009). Although donepezil treatment did not significantly slow the rate of hippocampal or ERC atrophy, a trend was noted toward slowing of the progression of total hippocampal atrophy and a significant slowing of right hippocampal atrophy in the post hoc analysis restricted to BSI-qualified scan pairs. Because selection of this subgroup was carried out blinded to treatment and chronological scan order, it could not have been driven by either of these factors. However, because this result was only detected by post hoc analysis and was not corrected for multiple comparisons, it must be considered tentative. Our study also showed that cognitive function, as measured by the modified ADAS-cog, correlated with whole brain volume measures at baseline and—most importantly—with respect to APC. The result is consistent with previous findings (Petersen et al., 2005) and further supports the view that atrophy rates measured with MRI are surrogate markers of disease progression. Taken together, these results imply a possible disease-modifying effect of donepezil in aMCI.

Several mechanisms have been identified by which cholinesterase inhibition might slow the progression of neuronal loss in aMCI. In vitro studies have found that muscarinic receptor stimulation decreases beta-amyloid production (Wolf et al., 1995) and that nicotinic receptor stimulation partially protects neurons from beta-amyloid-induced neurotoxicity (Svensson and Nordberg, 1998). Perhaps more clinically relevant is the hypothesis that ChEi treatment, by facilitating cholinergic neurotransmission, may promote maintenance of synaptic integrity and thereby resistance to neurodegeneration (Cummings, 2005). Recent functional MRI studies in subjects with MCI, showing that recruitment of the hippocampus and of other brain regions in response to a range of experimental tasks is significantly increased by ChEI treatment, are supportive of this hypothesis (Goekoop et al., 2004; Gron et al., 2006).

The greater impact of donepezil treatment on whole brain atrophy than on hippocampal or ERC atrophy is noteworthy, given that aMCI is considered to be, in most cases, a transitional stage to AD, and AD structural changes are thought to start in the ERC and hippocampus before spreading to cortical regions (Morris et al., 2001; Petersen et al., 2001). Interestingly, similar 1-year results were reported for rivastigmine in a large clinical trial in MCI, in which ventricular atrophy, but not ERC or hippocampal atrophy, was significantly less in the rivastigmine group compared with the placebo group ($p = 0.009$, uncorrected), though this difference was not present at 3 or 4 years (Feldman et al., 2007). It is possible that pathological factors are responsible

### Table 4

<table>
<thead>
<tr>
<th>Volume</th>
<th>Baseline</th>
<th>Study endpoint$^*$</th>
<th>% Rate of change per year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADAS-cog</td>
<td>CDR-SB</td>
<td>ADAS-cog</td>
</tr>
<tr>
<td>Total hippocampal</td>
<td>$-0.375^b$</td>
<td>$0.408^b$</td>
<td>$-0.127$</td>
</tr>
<tr>
<td>Left hippocampal</td>
<td>$-0.404^b$</td>
<td>$-0.061$</td>
<td>$-0.180$</td>
</tr>
<tr>
<td>Right hippocampal</td>
<td>$-0.328^b$</td>
<td>$-0.254^b$</td>
<td>$-0.154$</td>
</tr>
<tr>
<td>Total ERC</td>
<td>$-0.217^b$</td>
<td>$-0.101$</td>
<td>$-0.110$</td>
</tr>
<tr>
<td>Left ERC</td>
<td>$-0.203^b$</td>
<td>$-0.047$</td>
<td>$-0.166^b$</td>
</tr>
<tr>
<td>Right ERC</td>
<td>$-0.203^b$</td>
<td>$0.233^b$</td>
<td>$0.086$</td>
</tr>
<tr>
<td>Whole brain atrophy</td>
<td>$-0.115$</td>
<td>$0.136^b$</td>
<td>$0.140$</td>
</tr>
<tr>
<td>Ventricular region</td>
<td>$0.261^b$</td>
<td>$0.101$</td>
<td>$0.056$</td>
</tr>
<tr>
<td>Cortical region</td>
<td>$-0.155^b$</td>
<td>$-0.003$</td>
<td>$0.187^b$</td>
</tr>
</tbody>
</table>

Number of subjects: hippocampal, $n = 230$; ERC, $n = 213$ (baseline), $n = 212$ (endpoint and % rate of change/year); whole brain atrophy, ventricular, and cortical regions, $n = 214$ (baseline), $n = 164$ (endpoint and % rate of change/year).

Key: ERC, entorhinal cortex; CDR-SB, Clinical Dementia Rating-sum of boxes; ADAS-cog, modified Alzheimer Disease Assessment Scale-cognitive subscale (Doody et al., 2009; Rozzini et al., 2008); SC, Spearman correlation coefficient.

$^a$ For whole brain atrophy and ventricular and cortical regions, change from baseline to study endpoint.

$^b$ $p<0.0001$.

$^c$ $p=0.001$.

$^d$ $p<0.01$.

$^e$ $p<0.05$.
for this observation. For example, it is well documented that beta-amyloid is more widespread in cortical regions than in the hippocampus, especially at an early stage of the disease (Braak and Braak, 1996). Based on the hypothesis that donepezil may attenuate amyloid-induced neuronal toxicity (Svensson and Nordberg, 1998; Wolf et al., 1995), it is conceivable that the treatment has a greater effect on the cortex and whole brain in general than on the hippocampus. This would also explain the dominance of the effect on the whole brain despite observations that both treated and untreated patients showed greater atrophy progression in the hippocampus. However, it cannot be completely ruled out that methodological differences between measurements of whole brain and hippocampal atrophy rates are responsible for the observation. For example, power to detect a treatment effect is more limited for the hippocampus than for cortical regions because tracing the anatomical boundary of the hippocampus (and ERC) reliably is notoriously difficult. Furthermore, hippocampal and ERC tracings are based on soft segmentation that can exploit partial volume effects and may therefore capture subtle changes more effectively (Ezekiel et al., 2004). In fact, prior work in this field has shown the greater sensitivity of BSI for detecting whole brain atrophy in MCI as compared with focal measurements of hippocampal or ERC atrophy (Ezekiel et al., 2004; Misra et al., 2009; Spulber et al., 2008).

In contrast to the results of the present trial, a prior donepezil aMCI clinical study employing similar MRI volumetric analysis (Jack et al., 2008) did not find a treatment effect, even though MRI scan intervals in that study were more than twice as long. Several reasons may account for the discrepant findings. First, the sample size was larger in the present study (placebo, \( n = 90 \) versus \( n = 54 \); donepezil, \( n = 74 \) versus \( n = 37 \)). Second, a high degree of heterogeneity exists among patients diagnosed with aMCI, which may lead to important differences in the patient populations examined (Fleisher et al., 2007). Third, this MRI substudy was included in the initial protocol of the main study, whereas the prior MRI substudy was grafted on at a later date. Consequently, the methodological rigor employed in this study with respect to site qualification, scan acquisition, image quality control, and reader training and oversight was greater than in the previous study.

Stratifying subjects by APOE status did not result in the demonstration of a treatment effect, although APOE e4-positive subjects did demonstrate a higher rate of atrophy overall than the APOE e4-negative group, as has been observed previously (Hashimoto et al., 2005; Jack et al., 2008).

Modified ADAS-cog scores correlated significantly, but not very strongly, with all but one of the volumetric measures at baseline and endpoint, and with the APC for total, cortical, and ventricular whole brain atrophy, consistent with findings from a previous study of donepezil in aMCI (Jack et al., 2008). The CDR-SB, however, did not correlate well with volumetric measures at baseline, endpoint, or with respect to change. Moreover, in this trial and 2 preceding trials of donepezil in MCI, a significant treatment benefit was observed on the modified ADAS-cog, but not the CDR-SB (Doody et al., 2009; Petersen et al., 2005; Rozzini et al., 2008; Salloway et al., 2004). These findings suggest that donepezil treatment may have a greater effect on cognitive function than on global rating in aMCI.

This study has several limitations. First, the subjects have not been followed long enough to determine the incidence of incipient AD in each group. Thus, the difference in the rates of brain atrophy and cognitive decline between the study groups may partly reflect a difference in the proportion of subjects with preclinical AD within each group. Second, the cognitive tests and rating scales used in this study may not have been sufficiently sensitive to the subtle and slower changes characteristic of aMCI to provide a highly accurate assessment of change. Third, white matter lesions, an indication of cerebrovascular disease, have been shown to modulate hippocampal atrophy (Fein et al., 2000). It is therefore possible that the slowing of brain atrophy from treatment with donepezil may differ as a function of white matter lesions.

Overall, these findings suggest that donepezil has a treatment effect on whole brain volume changes in aMCI. The hippocampal volumetric results are more encouraging than those previously reported and suggest their possible utility as well. These results provide support for the use of whole brain MRI volumetric analysis in studying both the natural history of disease progression and therapeutic interventions in aMCI. The combined use of MRI brain volumetric measures with clinical rating scales and cognitive tests will allow for greater statistical power to detect change in studies of patients with aMCI.

**Disclosure statement**

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The study protocol was approved by the institutional review board of each site, and informed consent was obtained from all subjects.

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design, data collection, and manuscript content were conceived by the authors.

References

Mennier, J., Jeni, J., Maurice, T., 2006. The anti-amyloid and neuroprotective effects of donepezil against amyloid beta(25–35) peptide-induced toxicity in mice involve an interaction with the sigma(1) receptor. Br. J. Pharmacol. 149, 998–1012.


Online Supplement

Figure e-1. Magnetic resonance images from the training manual. (A) Entorhinal cortex (ERC) on coronal slice: representative T1-weighted magnetic resonance imaging (MRI) scan showing a coronal section through the ERC. The boundaries of this small brain structure, derived manually using anatomical landmarks, are highlighted in red (right ERC) and blue (left ERC). (B) Hippocampus on coronal and sagittal slices: representative T1-weighted MRI showing a coronal and sagittal section through the mesial temporal lobe including the hippocampus. The boundaries of the hippocampus, derived automatically by warping an atlas brain onto this individual brain image, are highlighted in red. (C) Pairs of whole brain scans with boundary shifts highlighted: results of boundary shift integral (BSI) analyses of 2 pairs of images acquired about 1 year apart. The BSI results on the left, lacking any observable feature, suggest that there has been no major volume change of the brain. The BSI results on the right, highlighting boundary shifts, indicate enlargements of ventricles and sulcal spaces, consistent with brain volume loss.

Table e-1
Percent rate of change per year in total hippocampal volume by ApoE genotype

<table>
<thead>
<tr>
<th>Treatment group (n)</th>
<th>ApoE positive</th>
<th>ApoE negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donepezil (23)</td>
<td>Placebo (31)</td>
</tr>
<tr>
<td></td>
<td>Donepezil (28)</td>
<td>Placebo (35)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$-2.06$ (3.935)</td>
<td>$-1.66$ (5.103)</td>
</tr>
<tr>
<td>Range</td>
<td>$-7.9$ to $4.6$</td>
<td>$-8.4$ to $19.7$</td>
</tr>
<tr>
<td>LS mean$^2$</td>
<td>$-2.63$</td>
<td>$-3.70$</td>
</tr>
<tr>
<td>SE$^2$</td>
<td>1.427</td>
<td>1.348</td>
</tr>
<tr>
<td>Treatment difference (95% CI)</td>
<td>1.07 (−1.66 to 3.81)</td>
<td>2.22 (−0.45 to 4.89)</td>
</tr>
<tr>
<td>$p$ Value</td>
<td>0.4319</td>
<td>0.1008</td>
</tr>
</tbody>
</table>

Key: ApoE, apolipoprotein E; BSI, boundary shift integral; CI, confidence interval; LS, least-squares estimated mean; SD, standard deviation; SE, standard error; (2): from a linear mixed-effects analysis.

*a The data presented in the table are limited to BSI-qualified scans. Thus, the number of samples is lower (placebo, 66; donepezil, 51) than the total number of samples with ApoE data (placebo, 92; donepezil, 74). The results in the full data set are no different (ApoE negative: $0.91 [-1.31$ to $3.13], p = 0.4164$; ApoE positive $1.65 [-1.17$ to $4.46] p = 0.2473$).
Table e-2

Linear mixed effects analysis\(^a\) of total hippocampal APC (restricted to BSI-qualified scans)

<table>
<thead>
<tr>
<th></th>
<th>Value [mm(^3)]</th>
<th>SE [mm(^3)]</th>
<th>df</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>4024.594</td>
<td>65.71662</td>
<td>160</td>
<td>61.24164</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Scan interval</td>
<td>−65.784</td>
<td>15.79048</td>
<td>160</td>
<td>−4.16602</td>
<td>0.0001</td>
</tr>
<tr>
<td>Treatment group</td>
<td>86.558</td>
<td>65.71662</td>
<td>160</td>
<td>1.31713</td>
<td>0.1897</td>
</tr>
<tr>
<td>Scan interval by treatment group interaction</td>
<td>−28.811</td>
<td>15.79048</td>
<td>160</td>
<td>−1.82456</td>
<td>0.0699</td>
</tr>
</tbody>
</table>

Random effects, intercept SD = 820.2 mm\(^3\), residuals = 138.7; fixed effects formula, Volume = Intercept + a1*Scan interval + a2*Treatment group + a3*Scan interval *Treatment group; here: Volume is total hippocampal volume; a1, a2, a3 are coefficients of the regression number of observations = 324; number of groups = 162. Bold text indicates treatment effect (the corresponding ANOVA p value for total hippocampal APC restricted to BSI-qualified scans was \(p = 0.076\) [Table 2, main text]).

Key: ANOVA, analysis of variance; APC, annualized percentage change; ApoE4, apolipoprotein E4; BSI, boundary shift integral; df, degrees of freedom; SD, standard deviation; SE, standard error.

\(^a\) The ANOVA tests of a treatment effect on brain volume changes were supplemented post hoc by linear mixed effects analyses, in which volumes were regressed against time as fixed effects separately from variations in baseline volumes as random effects. Other explanatory variables, e.g., treatment, age, or ApoE4 status, were added into the model as appropriate. To determine if the addition of explanatory variables, especially the inclusion of treatment, improved explanatory power, paired models were designed with and without the additional explanatory variable.

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**e-References**

