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Changes in Regional Cerebral Blood Flow and Functional Connectivity in the Cholinergic Pathway Associated with Cognitive Performance in Subjects with Mild Alzheimer's Disease after 12-week Donepezil Treatment

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Abstract

Acetylcholinesterase inhibitors (AChEIs), such as donepezil, have been shown to improve cognition in mild to moderate Alzheimer's disease (AD) patients. In this paper, our goal is to determine the relationship between altered cerebral blood flow (CBF) and intrinsic functional network connectivity changes in mild AD patients before and after 12-week donepezil treatment. An integrative neuroimaging approach was employed by combining pseudocontinuous arterial spin labeling (pCASL) MRI and resting-state functional MRI (R-fMRI) methods to determine the changes in CBF and functional connectivity (FC) in the cholinergic pathway. Linear regression analyses determined the correlations of the regional CBF alterations and functional connectivity changes with cognitive responses. These were measured with the Mini-Mental Status Examination (MMSE) scores and Alzheimer's disease Assessment Scale-Cognitive subscale (ADAS-cog) scores. Our results show that the regional CBF in mild AD subjects after donepezil treatment was significantly increased in the middle cingulate cortex (MCC) and posterior cingulate cortex (PCC), which are the neural substrates of the medial cholinergic pathway. In both brain regions, the baseline CBF and its changes after treatment were significantly correlated with the behavioral changes in ADAS-cog scores. The intrinsic FC was significantly enhanced in the medial cholinergic pathway network in the brain areas of the parahippocampal, temporal, parietal and prefrontal cortices. Finally, the FC changes in the medial prefrontal areas demonstrated an association with the CBF level in the MCC and the PCC, and also were correlated with ADAS-cog

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score changes. These findings indicate that regional CBF and FC network changes in the medial cholinergic pathway were associated with cognitive performance. It also is suggested that the combined pCASL-MRI and R-fMRI methods could be used to detect regional CBF and FC changes when using drug treatments in mild AD subjects.

Keywords

acetylcholinesterase inhibitors; donepezil; cerebral blood flow; resting-state functional connectivity; Alzheimer's disease

Introduction

Clinically, acetylcholinesterase inhibitors (AChEIs), [1] such as donepezil, have been approved to treat Alzheimer's disease (AD) patients with the goal of improving cognitive benefits. According to the "cholinergic hypothesis," disruption of the basal forebrain cholinergic pathways in the central nervous system significantly contributes to cognitive decline in advanced aging and AD (Bartus et al., 1982). The loss of cholinergic input to the cerebral neocortex and hippocampus results in a decreased acetylcholine (ACh) level, which is one of the principle neuromodulators of synaptic plasticity, including learning and memory (Buccafusco and Terry, 2000; Terry and Buccafusco, 2003). Hence, treatment of AD patients with donepezil could prevent the breakdown of ACh and enhance synaptic cholinergic transmission (Bartus, 2000). Clinical trials have shown that donepezil has modest effects on memory and cognitive improvement in responders (Black et al., 2007; Hansen et al., 2008; Pepeu and Giovannini, 2010; Rogers et al., 1998). However, the physiological mechanisms by which cholinergic treatments affect brain functions in specific regions are not fully understood. This is especially true of the action mechanism relative to donepezil at the brain system level.

Previous studies showed that heightened cerebral metabolism and cerebral blood flow (CBF) are essential in maintaining increased cholinergic activity (Terry and Buccafusco, 2003). Functional neuroimaging techniques, such as single-photon emission computed tomography (SPECT), positron emission tomography (PET) and arterial spin labeling (ASL) MRI, have been employed in AD research to measure treatment-mediated changes in regional CBF and cerebral metabolism in several distributed brain regions in the anterior cingulated cortex (ACC), posterior cingulate cortex (PCC), hippocampus, inferior parietal cortex (IPC) and frontal regions (Bohnen et al., 2005; Dai et al., 2009; Hanyu et al., 2010; Minoshima et al., 1997; Nakano et al., 2001; Staff et al., 2000; Tateno et al., 2008; Ushuijima et al., 2006; Yoshida et al., 2007). In particular, the CBF levels in the frontal brain regions are shown to be associated with AD treatment responses (Hanyu et al., 2003; Hongo et al., 2008). However, it is not fully understood why a similar donepezil treatment regime in different neuroimaging studies activated different brain regions. It is not known how these brain regions activated by donepezil treatment were interrelated.

In order to investigate interactions or interrelations between brain regions that responded to donepezil treatment, the resting-state functional connectivity MRI (R-fMRI) method was employed (Biswal et al., 1995). This method measures the temporal correlation between the spontaneous blood oxygenation level-dependent (BOLD) fluctuations in spatially separated,

[1]AChEI: acetylcholinesterase inhibitors, AD: Alzheimer's disease, CBF: cerebral blood flow, ASL: arterial spin labeling, pCASL: pseudocontinuous arterial spin labeling, R-fMRI: resting-state functional MRI, FC: functional connectivity, MMSE: Mini-Mental Status Examination, ADAS-cog: Alzheimer's disease Assessment Scale-Cognitive subscale, NPI: Neuropsychiatric Inventory, IADL: Instrumental Activities of Daily Living, MCC: middle cingulate cortex, PCC: posterior cingulate cortex, ACC: anterior cingulate cortex, IPC: inferior parietal cortex, vACC: ventral anterior cingulate cortex, VMPFC: ventral prefrontal cortex.

but functionally related, brain regions at resting-state, as extensively reviewed (Fox and Raichle, 2007). R-fMRI has been widely applied in investigating the functional network changes in normal and diseased human brains (Fox and Greicius, 2010), and it specifically has been applied to AD studies (Agosta et al., 2010; Buckner et al., 2009; Buckner et al., 2005; Chen et al., 2011a; Li et al., 2002a; Wang et al., 2006). In AD and other neuropsychiatric disorders, this modality also has been used to examine the relationship between neural network function changes and treatment outcomes (Goveas et al., 2011; Kozel et al., 2011; Lui et al., 2010). These studies suggest that R-fMRI is a valuable tool in examining changes in the intrinsic functional networks in targeted brain regions and the behavioral significance associated with pharmacotherapy.

In this study, our goal is to identify the impact of donepezil treatment on specific neural systems, thereby demonstrating cognitive benefit in patients with mild AD (Mini-Mental Status Examination score is larger than 23). To realize this goal, we employed an integrative neuroimaging approach by combining pseudocontinuous ASL (pCASL) MRI and the R-fMRI methods to determine the relationship between the altered CBF and the changes in the intrinsic functional network connectivity in the cholinergic pathway in mild AD before and after the 12-week donepezil treatment. We hypothesize that: 1) treatment of mild AD patients with donepezil will enhance the CBF level and functional connectivity in the cholinergic pathways; 2) the changes in the CBF level and functional connectivity strength in the cholinergic pathways are associated with changes in cognitive performance in subjects with mild AD. Our results demonstrate that treatment of mild AD subjects with donepezil primarily improves regional CBF and functional connectivity in the medial cholinergic pathway, which is associated with cognitive performance.

Materials and Methods

Participants

Fourteen patients with newly diagnosed mild AD (nine males and five females of age 77.6±7.1 years), who had never received any AD treatment medications, were recruited through Froedtert Hospital and the Medical College of Wisconsin Memory Disorders Clinic (Milwaukee, Wis., USA). The study was conducted with the approval of the Medical College of Wisconsin Institutional Review Board (IRB) and in compliance with the Health Insurance Portability and Accountability Act (HIPAA regulations). Written informed consent was obtained from all participants or caregivers. All patients underwent physical, psychiatric and neurological examinations before and after treatment. Participants with mild AD received the starting regimen of donepezil 5 mg/day for the first four weeks, and the dose was increased to 10 mg/day after four weeks. The baseline clinical visits occurred 14 ± 10 days prior to the baseline MRI scans for all participants. Each subject with mild AD received a baseline MRI scan on day 0 (AD_B) and another scan within five days of the last day of 12-week donepezil treatment (AD_T).

Inclusion and Exclusion Criteria

During the baseline clinical visit, mild AD was diagnosed in patients according to National Institute of Neurological Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984). Patients were examined by two experienced neurologists with expertise in dementia. They received a full clinical assessment, which included a neurological examination, Mini-Mental State Examination (MMSE), Alzheimer's disease Assessment Scale-cognitive subscale (ADAS-cog), the Lawton Instrumental Activities of Daily Living Scale (IADL), and Neuropsychiatric Inventory (NPI) (Cummings et al., 1994; Folstein et al., 1975; Lawton and Brody, 1969; Rosen et al., 1984). None of the subjects had previously taken any medications

that could affect the CBF. Exclusion criteria included history of claustrophobia, Hachinski Ischemia Scale score > 4, MMSE score ≤ 23, major psychiatric diagnoses, including primary psychotic and affective disorders, and other types of neurodegenerative or secondary dementias. Further criteria for exclusion included cardiovascular disease, a history of stroke, the presence of two or more lacunar infarcts of 5 mm each and the use of anticoagulants or other known vasoactive medications. No hypertension was observed in any subject at the time of the MRI scans. Two AD patients were excluded from the data analysis, because of incomplete scans. All behavioral data were analyzed with SPSS 16.0 software (http://www.spss.com/. Chicago, Ill.).

pCASL and R-fMRI Data Acquisition

All MRI scans were performed on a GE 3T Signa LX scanner (Waukesha, Wis.) with a standard quadrature transmit-receive head coil. Study subjects were asked to lie still with their eyes closed while in the scanner. First, high-resolution anatomical images were acquired using a 3D spoiled gradient-echo (SPGR) sequence with 144 continuous axial slices. The scan parameters were as follows: TE/TR/TI of 4/10/450 ms, flip angle of 12°, slice thickness of a 1 mm, matrix size of 256×192 and a field of view of 24×24 cm^2. The functional perfusion MRI scans lasted 6 minutes. During this time, 12 axial slices were acquired. Each had 7-mm thickness for each slice and 1-mm gap by pseudocontinuous ASL (pCASL) MR pulse sequence (Dai et al., 2008). A single-shot EPI pulse sequence was employed for image acquisition with the following parameters: TR of 4 s, TE of 25 ms, labeling duration/post inversion delay of 1.5/1.75 s and a matrix size of 64×64. The localization of the imaging area was selected manually in order to cover the bottom margin of the inferior temporal lobe to the inferior parietal lobe. The tagging slice was placed 32.5 mm below the inferior edge of the imaging slab. Sagittal R-fMRI datasets of the whole brain were obtained in 6 minutes with a single-shot gradient echo-planar imaging pulse sequence with the following parameters: TR of 2 s, TE of 25 ms, flip angle of 90°; slice thickness of 4 mm, and a matrix size of 64×64; 36 slices were obtained without gap between slices.

Measurement of Absolute CBF

The pCASL image of each subject was analyzed using Analysis of Functional NeuroImages (AFNI) software (http://afni.nimh.nih.gov/afn/) and Matlab Version 10 (The Math Works, Inc., Natick, Mass.). First, subject motion was corrected by performing a volume registration on the pCASL data set. Separation of the tagged and nontagged images was then performed by concatenating odd and even scans, respectively. The mean difference of the pCASL images (ΔM) for each subject was calculated as the difference between the tagged and nontagged images. Furthermore, the absolute CBF was quantified, as previously described (Buckner et al., 2009; Wang et al., 2005). The formula for the calculation of absolute CBF is provided below:

$$CBF = \frac{\lambda \Delta M}{2\alpha M_0 T1_a \left\{ e^{-\frac{\omega}{T1_a}} - e^{-\frac{(\tau+\omega)}{T1_a}} \right\}}$$

where $T1_a$ (0.61s) is the longitudinal relaxation time of blood, M_0 is the average image intensity of the control image, α is the labeling efficiency (85%) (Wu et al., 2007), τ and ω are the duration of the labeling pulse and postlabeling delay time, respectively. λ is the blood/tissue water partition coefficient (0.9 ml/g). Finally, the absolute CBF images for each subject were transformed to Talairach space through transformation matrix of SPGR high-resolution images using AFNI program (*adwarp),* and smoothed with a 10-mm Gaussian kernel for statistical comparisons. The differences in absolute CBF between AD_B and AD_T

groups were measured by a paired *t*-test ($p < 0.05$, corrected cluster size > 1028 mm^3). The associations between cognitive changes, such as the ΔADAS-cog, with the baseline CBF and the ΔCBF, after 12 weeks of donepezil treatment, were studied, using linear regression, after controlling any confounding effects of age and gender.

Functional Connectivity Analysis

Resting-state functional connectivity (FC) was calculated as the correlation between the spontaneous low-frequency fluctuations of the seed region of interest (ROI) and the whole brain (Fox et al., 2007). The data preprocessing procedure was carried out according to a previous study (Chen et al., 2011a). In brief, the spikes in raw R-fMRI data were removed, followed by the removal of the temporal signal trend and motion correlation. Furthermore, the potential confounding signals from the white matter (WM), cerebral spinal fluid (CSF), whole brain and motion were regressed out, using a general linear model. The cardiac aliasing (Glover et al., 2000) and the respiratory volume variation (Birn et al., 2006) were minimized, based on the cardiac pulse oxymeter signal and the respiratory belt signal using AFNI programs (*3dretroicor*). A band-pass filter was applied to keep the low-frequency fluctuations within 0.015 Hz and 0.1 Hz. Finally, all preprocessed images were spatially transformed to the Talairach space for group level analysis.

The seed regions were selected as the brain areas with significantly altered CBF after treatment. The average time course in each of the seeds was then extracted from the preprocessed R-fMRI data and cross-correlated with the entire brain voxel by voxel to calculate the Pearson product-moment correlation coefficient. Fisher's *z*-transform and spatial smoothing (6-mm Gaussian) then were applied to obtain the FC maps for each subject. Within-group FC network patterns and between-group FC network differences were calculated using a one-sample *t*-test ($p < 0.01$, corrected cluster size > 1672 mm^3) and paired *t*-test ($p < 0.05$, corrected cluster size > 4048 mm^3), respectively. Finally, linear regression analyses were performed to examine the relationships among changes in the FC (ΔFC), baseline CBF, ΔCBF and ΔADAS-cog, while controlling for the possible confounding effects of age and gender as covariates using a general linear model. All voxelwise multiple comparisons were corrected with 3dClusterSim program in AFNI software.

Results

Demographics and Clinical Evaluations

Twelve AD subjects completed the follow-up study and were included in the data analysis. Table 1 shows the demographic information and cognitive testing scores for AD_B and AD_T. There were no significant differences in age ($p = 0.992$) and gender ($p = 0.48$) between the two groups. After 12 weeks of donepezil treatment, the AD_T group had significantly improved ADAS-cog scores compared to the baseline measures ($p < 0.002$). However, no significant differences were found in the MMSE, NPI, and IADL scores between the two groups.

Enhanced CBF and its cognitive significance

Figure 1 shows the quantitative group-average CBF images from the mild AD group at baseline (top) and 12 weeks after donepezil treatment (bottom). Figure 2A shows that the CBF was significantly increased in the bilateral middle cingulate cortex (MCC) and posterior cingulate cortex (PCC) ($p < 0.05$, corrected) in the AD_T group, using a paired *t*-test between AD_B and AD_T groups. No other brain regions were found to have significant CBF changes. The MCC cluster center is located at the junction between BA 23 and 24 with the Talairach coordinates at x = -4 mm, y = -2 mm and z = 32 mm; the cluster size is 2064 mm^3. The center of the PCC cluster is located at BA 23 with the Talairach coordinates at x = -2

mm, y = -44 mm and z = 25 mm; the cluster size is 1624 mm^3. Figure 2B shows the significant changes in the CBF levels in the MCC and PCC regions between the AD_B and AD_T groups. Figure 2C illustrates the significant correlations between the ADAS-cog scores and CBF level in the MCC ($R^2 = 0.39$, $p < 0.03$) and the PCC ($R^2 = 0.34$, $p < 0.05$). The ΔCBF also was significantly correlated with the ΔADAS-cog in the MCC ($R^2 = 0.52$, $p < 0.009$) and the PCC ($R^2 = 0.46$, $p < 0.02$).

Enhanced FC in the MCC and PCC networks after treatment

Figure 3 shows the FC network patterns of the MCC (left column) and the PCC (right column) for AD_B (top row), AD_T (middle row) and the FC enhancement after treatment (bottom row), obtained using a one-sample *t*-test ($p < 0.01$, corrected) and a paired *t*-test ($p < 0.05$, corrected), respectively. The MCC connectivity network showed connections with the subcortical, parietal, frontal and insula brain regions (Table 2). The brain regions with significantly increased MCC connectivity were found in the bilateral ventral medial prefrontal cortex (VMPFC), ventral anterior cingulate cortex (vACC), left parahippocampal gyrus, right precuneus, right precentral gyrus and right inferior parietal cortex (IPC) (Table 4). No significantly decreased MCC connectivity was found. The PCC connectivity showed connections in the prefrontal, temporal and partial brain regions after treatment (Table 3). After treatment, the brain regions with significantly increased PCC connectivity were found in the bilateral vACC and right cuneus (Table 4). A significantly decreased FC between the PCC and anterior insula was found in the right cerebral cortex.

Correlations between changes in ADAS-cog, CBF and the changes in FC strength

In Figure 3, the **Δ**ADAS-cog score was significantly correlated with the **Δ**FC between the MCC and vACC ($R^2 = 0.44$, $p < 0.0192$), and between the MCC and the VMPFC ($R^2 = 0.36$, $p < 0.0377$) (Figure 4, top). The **Δ**FC between the MCC and the vACC was also significantly correlated with the baseline CBF level in the MCC ($R^2 = 0.7$, $p < 0.0007$). In the PCC connectivity network (Figure 4, bottom), the **Δ**FC between the PCC and the vACC was significantly correlated with the **Δ**ADAS-cog scores ($R^2 = 0.44$, $p < 0.0196$), baseline CBF ($R^2 = 0.76$, $p < 0.0003$) and the ΔCBF ($R^2 = 0.44$, $p < 0.019$) in the PCC.

Discussion

The results from this integrated pCASL-MRI and R-fMRI study demonstrate that cognitive performance in mild AD after 12-week donepezil treatment is associated with the alterations in the regional CBF and intrinsic functional networks. This conclusion is supported by three major experimental findings. First, regional CBF is significantly increased in the MCC and the PCC in the AD_T group when compared to the AD_B group. Second, the FC networks involving the MCC and the PCC are significantly enhanced in the AD_T group when compared to the AD_B group. Third, changes in the regional CBF and the FC networks are associated with the changes of cognitive performance as measured by the ADAS-cog scores.

Our results show increased regional CBF in the MCC and the PCC (Figure 2) after donepezil treatment. The previous study reports that the CBF of the posterior cingulate regions was significantly decreased in AD subjects compared with cognitively normal subjects (Bohnen et al., 2005; Dai et al., 2009; Hanyu et al., 2010; Minoshima et al., 1997; Nakano et al., 2001; Staff et al., 2000; Tateno et al., 2008; Ushuijima et al., 2006; Yoshida et al., 2007). Our findings suggest that an increased CBF in the PCC is reflective of an improvement of the cerebral blood flow values toward normalcy after donepezil treatment. These results also are consistent with the “cholinergic hypothesis” (Bartus, 2000). It is conceivable that treatment of AD with AChEIs should restore, or partially restore, synaptic activities within the cholinergic pathways, and the CBF increase reflects elevated brain

metabolism and neural activity among brain regions within these cholinergic pathways. Intriguingly, the MCC and the PCC are the two most important regions located in the medial cholinergic pathway (MCP), a major pathway in human cholinergic networks (Selden et al., 1998). Our significant finding is that in using the R-fMRI technique most of the isolated brain regions that showed increased CBF in previous perfusion studies (Nakano et al., 2001; Staff et al., 2000; Tateno et al., 2008; Ushuijima et al., 2006; Yoshida et al., 2007) were functionally interconnected to the MCC and the PCC in the MCP pathway. These brain regions include the PCC, vACC, parietal cortices, MTG and VMPFC (Tables 2 and 3). We found that the FC of the parahippocampal gyrus, vACC, IPC and VMPFC connected to the MCC and the PCC were partially recovered after treatment in patients with mild AD (Table 4). More importantly, the changes in the FC were significantly correlated with the cognitive performance and the regional CBF in the MCC and the PCC (Figure 4). Furthermore, as a previous study reported, the connectivity increase of the posterior cingulate regions was significantly decreased in AD subjects compared with cognitively normal subjects (Buckner et al., 2009; Greicius et al., 2004). To address the question of PCC and MCC connectivity in control subjects, we employed and analyzed R-fMRI datasets previously acquired from age-matched cognitively healthy controls (12 subjects). The PCC and MCC connectivity in the control subjects was significantly higher than that in AD subjects. Therefore, the increased connectivity after treatment, as shown in Figure 3, could be interpreted as the improvement toward normalcy.

The MCP, in addition to the lateral cholinergic pathway, is a primary white matter pathway in the human cholinergic network. The MCP trajectory originates at the nucleus basalis of Meynert of the basal forebrain and extends medially through the cingullum and corpus callosum, primarily innervating the neocortex (Selden et al., 1998). Although the function of the MCP is not completely understood, it has been suggested that the posterior parts of the cingulate cortices, such as the MCC and the PCC, perform evaluative functions for spatial orientation and memory (Vogt et al., 1992). In animal studies, the septocingulate pathway, in which cholinergic neurons of the basal forebrain innervate the cingulate cortex, is critically involved in the working/episodic memory of rats (Dougherty et al., 1998). Recently, evidence from structural studies which used diffusion tensor imaging illustrates that the MCC and the PCC are an integral part of the core structures in the human cerebral cortex (Hagmann et al., 2008). These brain regions, which include the MCC, PCC, medial prefrontal lobe, middle temporal lobe and IPC, are hubs of the “structural highways” in the brain that interconnect functionally specialized networks (Buckner et al., 2009; Gong et al., 2009). Several studies indicate that disruption of networks that originate from these hub brain regions is associated with memory deficits and cognitive decline in dementia (Seeley et al., 2009; Xie et al., 2011). Thus, our findings of increased regional CBF and the enhancement of the underlying FC networks of the MCC and the PCC support the notion that donepezil treatment mediates positive synaptic changes in the cholinergic pathways in the AD brain. In turn, this will likely increase the corticocortical connection between the functionally interconnected brain regions. As a result, this enhanced connectivity in the neurocognitive networks may be responsible for the cognitive responses in patients following treatment.

Our results highlight the value and importance of examining donepezil-mediated effects on neurocognitive networks encompassing multiple interconnected brain regions in AD. It has been increasingly recognized that cognitive outputs are not likely to be mediated by individual cortical or subcortical brain regions. Rather, cognitive outputs are dependent on regions, which are interconnected and organized in ways that can facilitate synchronous neural activation, thereby forming the neurocognitive networks (Mesulam, 2009). Therefore, depending on the brain regions and the extent of their involvement in the neurocognitive networks, varying types and degrees of dysfunction can exist in the diseased brain. Recent

studies have linked the different categories of neurodegenerative diseases to dysfunctions in the distinct and specific brain functional networks (Seeley et al., 2009). A number of studies also have shown that cognitive changes in patients with neurodegenerative diseases are more likely the result of changes in the functional neural networks containing multiple brain regions rather than isolated regions (Kozel et al., 2011; Xie et al., 2011). Hence, cognitive improvement following donepezil treatment suggests that the drug targets and affects functional systems or networks involving multiple brain regions. It enhances the cognitive outcomes by increasing the connectivity between these anatomically separated regions. These findings suggest that the multimodal MRI techniques utilized in the current study are powerful tools for studying the efficacy of drug treatment in the regional and system levels in mild AD.

Several limitations of the current study should be considered when interpreting the results. First, the American Academy of Neurology views AChEI as the standard of clinical care. As a result, the IRB did not allow a placebo-controlled arm for this study. We were unable to evaluate any possible placebo effect of donepezil. However, previous studies have demonstrated that there has been no significant placebo benefit found in several donepezil trials in respect to regional CBF changes (Rogers et al., 1998; Yoshida et al., 2007) and cognitive changes (Black et al., 2007). Therefore, the likelihood that the observed CBF changes are because of a placebo effect is minimal. Second, the pCASL-MRI employed in this study acquired axial images from the upper part of the inferior parietal lobe to the inferior part of the temporal lobe, but not the whole brain. Third, the susceptibility artifacts from thicker imaging slices (Li et al., 2002b) rendered a low signal-to-noise ratio. We were unable to detect CBF changes in the orbitofrontal cortex where previous SPECT studies showed positive findings (Hanyu et al., 2003; Hongo et al., 2008). In future studies, fast-imaging acquisition methods, such as the multislice excitation method (Feinberg et al., 2010; Jesmanowicz et al., 2011) and the latest parallel imaging techniques, will be used to acquire thinner slices with full-brain coverage in order to provide complete characterization of whole-brain CBF. Finally, the present study represents a small cohort for a treatment study, although recent studies have demonstrated that pCASL is highly reproducible with a relatively small sample size of 10 subjects (Wang et al., 2011), 12 subjects (Chen et al., 2011b), and 22 subjects (Xu et al., 2010). It will be necessary to include more subjects in future treatment studies to achieve a more adequate statistical power.

In summary, the pCASL-MRI and R-fMRI techniques revealed that the 12-week donepezil therapy enhances regional CBF in the MCC and the PCC, and improves the underlying functional connectivity to the whole brain in mild AD. Linear regression analysis further demonstrated that the treatment-induced cognitive changes are associated with the changes in regional CBF and the underlying functional connectivity in these patients. This combined method is able to detect the regional CBF changes in the isolated brain regions. In addition, it provides clues as to how these segregated brain regions are interconnected as a whole to form integrated functional brain networks. Hence, the current strategy may provide a new approach in studying treatment efficacy during the course of clinical treatment.

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Highlights

We study the treatment effects of donepezil in mild Alzheimer's disease.

We use integrative MRI approach to examine functional changes after treatment.

Treatment improves regional cerebral blood flow in the cingulate cortices.

Treatment improves functional connectivity in the medial cholinergic pathways.

Cognitive performances are correlated with changes in blood flow and connectivity.

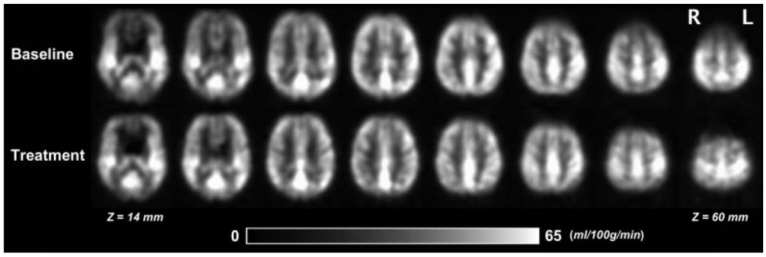

Figure 1.
Quantitative group-average CBF images from the mild AD group at baseline (**top**) and the same mild AD group 12 weeks after donepezil treatment (**bottom**).

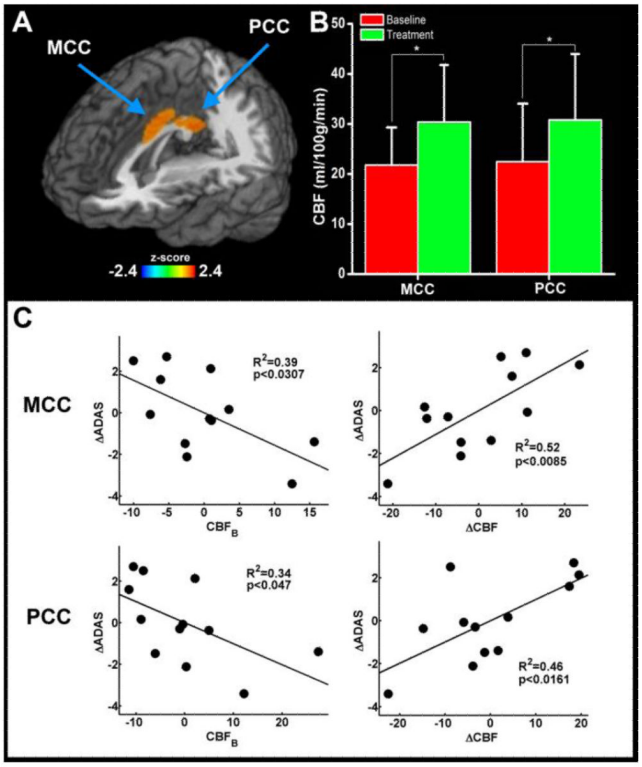

Figure 2. Effects of 12-week donepezil treatment on CBF levels and cognitive performance in mild AD patients

(**A**) The locations of MCC (BA 24) and PCC (BA 23) regions, as indicated by the arrows. (**B**) MCC and PCC showed increased CBF levels after donepezil treatment ($p < 0.05$). (**C**) The ΔADAS-cog scores and CBF at baseline, and ΔCBF in the MCC and PCC are significantly correlated after 12 weeks of donepezil treatment. The effects of individual subject age and gender variations were removed from the CBF and ADAS-cog measures. MCC=middle cingulate cortex, PCC=posterior cingulate cortex, ADAS-cog=AD assessment scale-cognitive subscale, Δ=changes.

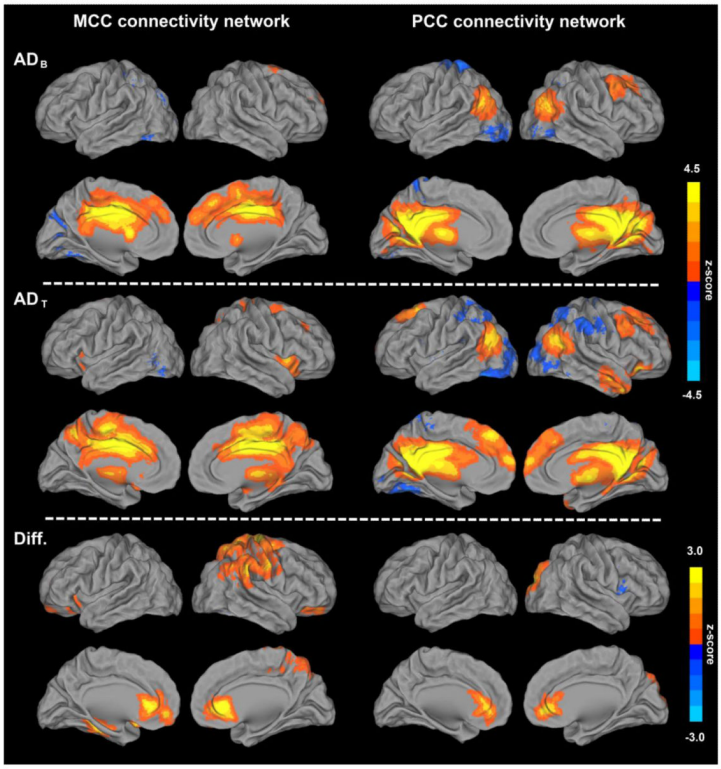

Figure 3. Illustrations of functional connectivity network patterns of MCC and PCC before and after 12-week donepezil treatment in mild AD patients

Compared to baseline connectivity patterns of the MCC and the PCC (**top** panel), donepezil treatment leads to enhanced functional connectivity in both regions (**middle** panel). Connectivity patterns before and after treatment were analyzed with one-sample *t*-test, and the details are shown in Tables 2 and 3, respectively. Bright colors indicate positive correlations, while blue colors indicate negative correlations. Brain regions that show significantly altered FC after donepezil treatment with paired *t*-test (**bottom** panel) are listed in Table 4.

MCC=middle cingulate cortex, PCC=posterior cingulate cortex.

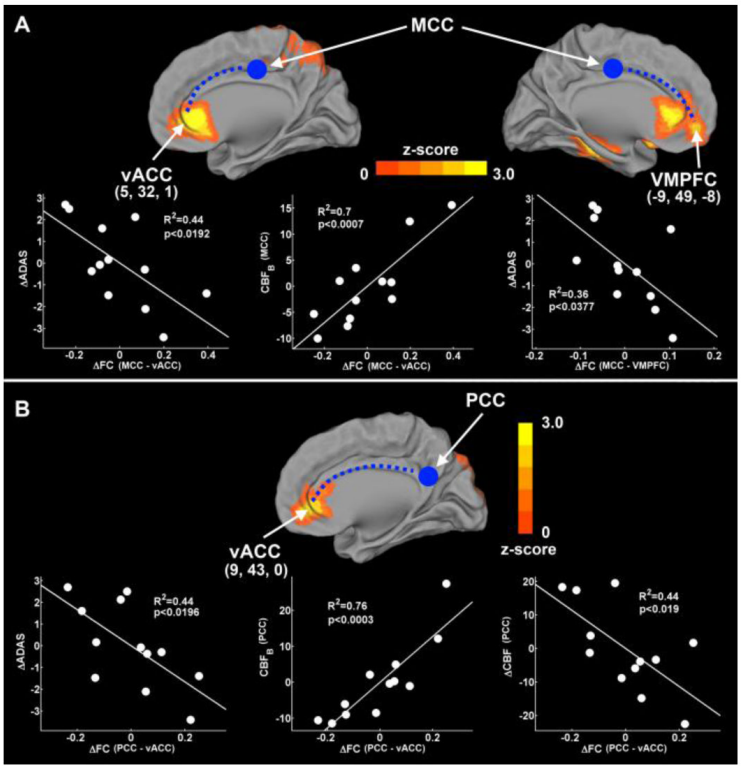

Figure 4. Behavioral significance of CBF, ΔCBF, and ΔFC in the MCC and PCC connectivity networks

In the MCC connectivity network (**A**), the **Δ**FC in the vACC and the VMPFC was significantly correlated with the **Δ**ADAS-cog scores. The **Δ**FC in the vACC was also significantly correlated with the baseline CBF in the MCC. In the PCC connectivity network (**B**), the **Δ**FC in vACC was significantly correlated with the **Δ**ADAS-cog scores, baseline CBF, and ΔCBF in PCC. The blue solid circles and dotted lines represent the seed regions and the functional connections, respectively.

vACC=ventral anterior cingulate cortex, VMPFC=ventral prefrontal cortex, MCC=middle cingulate cortex, PCC=posterior cingulate cortex, ADAS-cog=AD assessment scale-cognitive subscale, Δ=changes.

Table 1

Demographics and Clinical Evaluations

	Mild AD (N = 12)		*p*-value
	Baseline	**Treatment**	
Gender	4F/8M		0.480
Age	77.6±7.1	77.9±7.2	0.992
MMSE	26.8±1.4	26.5±2.7	0.852
IADL	13.1±3.8	12.7±3.7	0.941
ADAS-cog	12.0±4.6	9.78±4.8	0.002*
NPI	9.2±10	5.9±9.5	0.140

Note. – F: female, M: male, MMSE: Mini-Mental State Examination, ADAS-cog: Alzheimer's disease Assessment Scale-cognitive Subscale, NPI: Neuropsychiatric Inventory, IADL: Instrumental Activities of Daily Living,

*Statistically significant with paired *t*-test.

Table 2

Functional connectivity patterns of MCC and PCC networks before donepezil treatment in mild AD subjects

	Regions	BA	Cluster size (mm^3)	Talairach coordinates (LPI)			Z values
				X	Y	Z	
MCC connectivity network							
L	MCC (seed ROI)	24	29200	−4	−2	32	5.18
L	Caudate			−6	9	7	3.74
R	SFG	6		10	9	53	3.73
R	Caudate			5	6	0	3.25
L	MFG	9	2552	−3	46	25	3.41
R	MFG	9		7	50	22	2.89
L	Cuneus	18, 19	9856	−13	−77	29	−3.39
PCC connectivity network							
L	PCC (seed ROI)	23	65200	−2	−44	25	5.76
L	Thalamus			−3	−17	5	4.43
R	Thalamus			6	−15	7	4.29
R	MTG	39	6456	46	−63	21	3.56
L	MTG	39	6152	−49	−63	22	3.49
R	MFG	8	3864	22	25	42	3.29
L	Fusiform	18	13120	−26	−86	−12	−4.12
R	Fusiform	37		48	−58	−11	−3.18
L	Postcentral gyrus	3	1688	−18	−36	63	−3.32

Note. – BA: brodmann area, MCC: middle cingulate cortex, MeFG: medial frontal gyrus, SFG: superior frontal gyrus, PCC: posterior cingulate cortex, MTG: middle temporal gyrus, MFG: middle frontal gyrus

Table 3

Functional connectivity patterns of MCC and PCC networks after donepezil treatment in mild AD subjects

	Regions	BA	Cluster size (mm^3)	Talairach coordinates (LPI)			Z values
				X	Y	Z	
MCC connectivity network							
L	MCC (seed ROI)	24	40816	−4	−2	32	4.96
L	vACC	32		−7	27	24	3.95
L	Caudate			−12	10	8	3.06
L	Precuneus	7		−9	−52	45	4.57
R	ACC	32		8	28	24	3.31
R	Precuneus		7	9	−63	37	3.57
L	Putamen		10888	−19	6	10	4.35
R	Putamen		4880	26	6	−2	3.41
R	Insula	13		37	13	2	3.07
L	Fusiform	19	5496	−37	−70	−11	−3.22
PCC connectivity network							
L	PCC (seed ROI)	23	50704	−2	−44	25	5.31
L			Thalamus	−4	−21	9	4.38
R	Thalamus			6	−15	8	3.15
L	MFG	8	15992	−2	38	41	4.88
L	VMPFC	10		−2	56	14	3.93
L	vACC	32		−8	37	24	2.92
R	MFG	8		28	19	38	4.44
R	VMPFC	10		4	61	12	3.49
L	MTG	39	8152	−44	−64	18	3.73
R	MTG	39	6576	44	−64	25	4.39
R	STG	21, 38	3656	46	11	−29	3.49
L	Cuneus	17	12064	−16	−86	10	−3.58
R	Cuneus	17		20	−81	11	−2.78
R	SPC	7	9728	25	−60	47	−3.21
R	Postcentral gyrus	3		55	−23	39	−3.32

	Regions	BA	Cluster size (mm^3)	Talairach coordinates (LPI)			Z values
				X	Y	Z	
L	SPC	7	4928	−26	−58	47	−3.66

Note. – BA: brodmann area, MCC: middle cingulate cortex, vACC: ventral anterior cingulate cortex, PCC: posterior cingulate cortex, MTG: middle temporal gyrus, MeFG: medial frontal gyrus, VMPFC: ventral medial prefrontal cortex, SPC: superior parietal cortex, MTG: middle temporal gyrus, STG: superior temporal gyrus, MFG: middle frontal gyrus.

Table 4

Brain regions with significantly altered functional connectivity to MCC and PCC after donepezil treatment in mild AD subjects

	Regions	BA	Cluster size (mm^3)	Talairach coordinates (LPI)			Z values
				Z	X	Y	
MCC connectivity network							
R	vACC	24, 32	11080	5	32	1	3.98
L	VMPFC	10,11		−9	49	−8	2.84
R	Precuneus	7	7128	17	−45	56	3.44
R	Precentral gyrus	4	4560	18	−23	65	3.47
R	IPC	40		61	−34	29	2.86
L	Parahippocampus	35, 36	2040	−30	−26	−16	3.11
PCC connectivity network							
R	vACC	32	2440	9	43	0	2.92
R	Cuneus	19	2400	21	−82	32	3.25
R	Insula	13	1816	43	11	7	−3.20

Note. – BA: brodmann area, vACC: ventral anterior cingulate cortex, VMPFC: ventral media prefrontal cortex, IPC: inferior parietal cortex