Cholinergic enhancement increases regional cerebral blood flow to the posterior cingulate cortex in mild Alzheimer's disease

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Aim: The brain region that shows reductions in regional cerebral blood flow (rCBF) earliest is the posterior cingulate cortex (PCC), which is thought to have a relationship with cognitive function. We made a hypothesis that the PCC hypoperfusion is a result of cholinergic dysfunction and can be restored by cholinergic enhancement. This present longitudinal study aimed to detect the restoration of PCC rCBF in response to donepezil, an acetylcholine esterase inhibitor.

Methods: We evaluated rCBF changes in the PCC, precuneus and anterior cingulate cortex using perfusion single-photon emission computed tomography (SPECT), statistical analysis and region of interest analysis, prospectively. We allocated 36 patients with mild AD to either the responder or non-responder groups based on changes in Mini-Mental State Examination scores. The patients were followed up for 18 months.

Results: The PCC rCBF significantly increased in responders after 6 months of donepezil therapy. Statistical maps at baseline showed a typical decreased pattern of mild AD and obvious rCBF restoration in the bilateral PCC at 6 months in responders. Changes in Mini-Mental State Examination scores and the AD assessment scale cognitive scores significantly correlated with rCBF changes in the PCC of responders.

Conclusions: Cholinergic enhancement restored PCC rCBF under the three conditions of mild AD, responders and short follow-up interval, and that increase correlated with improved cognitive function. These findings support our hypothesis that PCC rCBF reflects cholinergic function in AD patients. Geriatr Gerontol Int 2017; 17: 951–958.

Keywords: Alzheimer's disease, donepezil, mild cognitive impairment, N-isopropyl-p-[¹²³I] iodoamphetamine, posterior cingulate cortex.

Introduction

The posterior cingulate cortex (PCC) is of particular interest, because it has dense structural connections to many other brain regions and could serve as a hub, and it is metabolically active with high regional cerebral blood flow (rCBF) in healthy individuals.¹–³ The region is thought to play an important role in cognition, although consensus about the nature of its role has not been reached.⁴ Reductions in rCBF and in the regional cerebral metabolic rate of glucose (rCMRglc) in the PCC comprise the earliest signs of Alzheimer's disease (AD).⁵,⁶ The mechanism of rCBF reduction in PCC is not still established. We made a hypothesis that decreased PCC rCBF is due to integration of cholinergic dysfunction, and is related to the impaired learning and memory associated with early AD.

If the PCC is indeed associated with the symptoms of AD, rCBF in the area should be restored with the use of an acetylcholine esterase inhibitor, such as donepezil. However, previous longitudinal single-photon emission computed tomography (SPECT) studies where donepezil has been administered have not shown an increase in PCC rCBF (Table 1).⁷–¹⁴

The present SPECT follow-up study aimed to determine whether PCC rCBF increases in response to donepezil and if so, to identify the optimal conditions for this to occur. As the PCC is one of the most vulnerable regions in AD and it is affected early during the course of the disease, we did not expect an increase in rCBF in this
Table 1  Single-photon emission computed tomography follow-up studies of donepezil therapy

| Reference | Tracer | Image analysis | Treated patients (n) | Baseline MMSE | Follow-up MMSE | Subgroup analysis | Follow-up imaging | Regions with increased or preserved rCBF |
|-----------|--------|----------------|---------------------|---------------|---------------|------------------|------------------|----------------------------------------|
| Staff et al.\textsuperscript{7} | \textsuperscript{99}Tc-HMPAO 3D-ROI | 12 | Not referred | Not referred | No | 35w | Overall increase in global CBF (most prominent in frontal lobes) |
| Nakano et al.\textsuperscript{8} | \textsuperscript{99}Tc-ECD SPM | 15 | 22.1 ± 3.3 | 19.9 ± 4.4 | No | 12m | Significantly preserved in bilateral ACC, right middle temporal gyrus, inferior parietal lobe and prefrontal lobe |
| Nobili et al.\textsuperscript{9} | \textsuperscript{99}Tc-HMPAO SPM | 25 | 19.8 ± 3.5 | 17.8 ± 4.1 | No | 11 ± 2.6m | No significant increase in any region |
| Shimizu et al.\textsuperscript{10} | \textsuperscript{123}I-IMP 3D-SSP SEE | 41 | 20.3 ± 4.1 | 23.8 ± 4.5 | Yes | 11.8 ± 1.3m | Significant increase in lateral and medial frontal lobes and orbital surface |
| Ushijima et al.\textsuperscript{11} | \textsuperscript{123}I-IMP 3D-SSP | 17 | 21.2 ± 4.9 | 22.5 ± 3.5 | No | 3m | Significant increase in relative rCBF in the frontal, parietal and temporal lobes |
| Yoshida et al.\textsuperscript{12} | \textsuperscript{123}I-IMP 3D-SRT | 29 | 18.5 | Not referred | Yes | 1m | Significant increase in anterior frontal lobe and parietal lobe |
| Tateno et al.\textsuperscript{13} | \textsuperscript{99}Tc-ECD 3D-SRT | 15 | 20.9 ± 4.7 | 18.7 ± 5.7 | No | 55.1 ± 11.0w | Significant increase in left callosomarginal, right central, bilateral pericallosal and lentical nucleus segments |
| Kimura et al.\textsuperscript{14} | \textsuperscript{99}Tc-ECD 3D-SRT | 31 | 20.7 ± 5.1 | 21.6 ± 4.8 | Yes | 24.5 ± 4.2m | No significant increase in any region |

ECD, ethyl cysteinate dimer; d, day; HMPAO, hexamethylpropyleneamine oxime; IMP, N-isopropyl-p-iodoamphetamine; m, month; n, number of patients; rCBF, regional cerebral blood flow; ROI, region of interest; SEE, stereotactic extraction estimation; SPM, statistical parametric mapping; SRT, stereotaxic ROI template; SSP, stereotactic surface projections; w, week.
region of advanced AD patients in response to donepezil. Therefore, a higher initial Mini-Mental State Examination (MMSE) score would be essential for the selection of patients who would be likely to benefit from and respond to donepezil with increased PCC rCBF. Furthermore, it was hypothesized that a rCBF increase in the PCC is more likely to manifest in responders presenting with cognitive improvements on donepezil therapy and that, inversely, non-responders might show limited augmentation of rCBF in the region. Considering its role in cognition, an increase of PCC rCBF would have considerable clinical importance.

Methods

Patient selection

We carried out a prospective study. All procedures adhered to the clinical study guidelines of Fukujuji Hospital, Tokyo, Japan, and were approved by the hospital ethics review board. Patients or their families provided written informed consent for the present study to enroll 36 consecutive outpatients attending Fukujuji Hospital (16 men and 20 women; age 69–89 years; mean age 77.6 years) between 2011 and 2012. The patients were diagnosed with probable mild AD according to the diagnostic criteria of the National Institute of Neurological and Communicative Disorders and Strokes–Alzheimer’s Disease and Related Disorders Association, the Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision and the International Classification of Disease, World Health Organization, 10th Revision. Major cerebral infarction was not seen on magnetic resonance imaging from any of the patients, and all of them had Hachinski Ischemic Scores ≤5, confirming that cerebrovascular factors were not involved in the pathophysiology of the disease. Scores on the MMSE, which is used to screen for dementia, ranged from 21 to 26 (mean 23.6). None of the patients used tranquilizers, anti-anxiety agents or antidepressants that would affect the central nervous system. The patients were given donepezil orally (3 mg/day for 2 weeks, followed by 5 mg/day for 18 months). All patients tolerated the higher dose without serious adverse reactions, and cohabiting family members confirmed their compliance. The MMSE and the Japanese version of the AD assessment scale-cognitive scale (ADAS-Jcog) were assessed at baseline, and at 6 and 18 months later. The patients were allocated to responder or non-responder subgroups based on an increase of ≥1 or a decrease of ≥2 compared with baseline MMSE scores at 6 months after therapy. According to Doody et al., the mean annual change in the MMSE scores of patients with untreated AD is −3.7 ± 4.6 (mean ± SD). As an annual MMSE score change of 0.9 (mean ± SD) as a cut-off was adequate, half of this value seemed suitable for an interval of 6 months. However, we selected a score of 1 as a cut-off for changes in MMSE scores, because the scores change by 1 point. We also included nine age-matched healthy controls defined as being free of cognitive complaints, and having a clinical dementia rating (CDR) of 0 and MMSE scores ≥28.

Brain perfusion SPECT imaging

Resting patients with closed eyes and unplugged ears were assessed using N-isopropyl-p-[123I] iodoamphetamine (123I-IMP) and an E-CAM gamma camera (Toshiba Medical Systems Corporation, Otawara, Japan) with fan beam collimators at baseline, and after 6 and 18 months of donepezil therapy initiation. From 15 min after an intravenous infusion of 167 MBq of 123I-IMP, SPECT images were acquired in a 128 × 128 matrix with a slice thickness of 1.95 mm (1 pixel) over a period of 30–40 min. The images were reconstructed using filtered back projection with Butterworth filter, attenuation was corrected using Chang’s method (attenuation coefficient = 0.1 cm⁻¹) and scatter was corrected with the triple energy window method.

Image analyses

Three-dimensional stereotactic surface projections (3D-SSP) created with Neurological Statistical Image Analysis Software (NEUROSTAT) developed by Minoshima et al. were applied to the 123I-IMP SPECT images to generate Z-score maps. Two-sample t-test values compared on a pixel-by-pixel basis between healthy controls and responders or non-responders (at baseline, and at 6 and 18 months) were transformed into Z-scores by probability integral transformation using NEUROSTAT. The rCBF distribution at baseline between the responders and the non-responders was compared. Statistical maps of changes in rCBF between values at baseline and at 6 months were also generated. We applied region of interest (ROI) analysis to measure PCC rCBF together with the adjacent precuneus and in the anterior cingulate cortex (ACC) using stereotaxic extraction estimation (SEE; version 2.1) software (Nihon Mediphysics, Tokyo, Japan). The precuneus and the ACC were selected as reference regions, because they are closely connected with PCC, and are less vulnerable than the PCC in mild AD in terms of rCBF decrease. The mean rCBF in each segment was automatically measured after segmentation based on anatomical classification of the standard brain (Fig. S1). We selected segments of the PCC (BA23 and BA31) and ACC (BA24) using Brodmann’s area (BA) level. We defined the boundary of the precuneus using gyrus level instead of BA7, considering that the precuneus is the mesial extent of BA7, which also contains several other regions. Relative CBF was determined by dividing the accumulation in each segment by global mean.
**Statistical analysis**

We used a one-way ANOVA to assess mean age and years of education among three groups. We applied the Tukey–Kramer test to correct the multiple comparison findings of changes in MMSE and ADAS-Jcog scores. Changes in relative CBF between baseline and 6 months were calculated using a paired t-test, with the Bonferroni correction. Correlations between rCBF changes in each segment and changes in MMSE or ADAS-Jcog scores were analyzed using the Pearson product-moment correlation coefficient.

**Results**

**Demographic characteristics**

Table 2 compares the demographic features of the patients with mild AD and healthy controls. Age and years of education did not significantly differ among the three groups. The MMSE scores at baseline also did not significantly differ among responders, non-responders and healthy controls. Mean MMSE and ADAS-Jcog scores were significantly improved at 6 months after baseline in the responders ($P < 0.05$), but deteriorated at 18 months.

The decline in mean MMSE and ADAS-Jcog scores between baseline and 18 months was consistent in the non-responders (Fig. S2).

The rCBF distribution at baseline was not significantly different between the responders and the non-responders (Fig. S3).

**Changes in rCBF between baseline and 6 months later in patients with mild AD**

We compared relative CBF in the bilateral PCC (BA23 and BA31), precuneus, and ACC (BA24) in segments of 3D brain images acquired at baseline and 6 months later from all patients (Table 3). Regional CBF was elevated in the left BA23 and bilateral BA31 ($P < 0.01$) and in the right BA23 ($P < 0.05$).

**Statistical maps of responders compared with healthy controls**

A comparison of rCBF on 3D-SSP images between responders and healthy controls showed relative hypoperfusion in the bilateral PCC, precuneus, right parietal lobe and left orbitofrontal cortex at baseline in the responders (Fig. 1). This distribution of decreased rCBF was typical of mild AD. Six months...
later, although relative hypoperfusion was evident in the bilateral PCC, left precuneus and right parietal lobe, the findings were less remarkable than at baseline. At 18 months, rCBF was obviously decreased in the bilateral PCC, precuneus and parietal lobe, findings that were typical of AD.

Statistical maps of non-responders compared with healthy controls

Comparisons of rCBF on 3D-SSP images from non-responders and healthy controls showed relative hypoperfusion in the bilateral PCC, precuneus, parietal lobe and temporal lobe at baseline of non-responders (Fig. 1). These findings became more remarkable at 18 months later.

Statistical maps of rCBF changes between baseline and 6 months in responders

Surface images of relative changes in rCBF between baseline and 6 months showed increased rCBF in the bilateral PCC, precuneus, ACC, thalamus and right dorsolateral prefrontal cortex of responders with mild AD (Fig. S4a). There was a significant increase in rCBF in the rostral region of the bilateral PCC. Peak Z was observed in the PCC (Talairach coordinates: x = −4; y = −30; z = 34; Z-score, 4.36; Fig. S4b).

Statistical maps of rCBF changes between baseline and 6 months in non-responders

Surface images of relative changes in rCBF between baseline and 6 months showed increased rCBF in the bilateral ACC, thalamus and right dorsolateral prefrontal cortex of non-responders with mild AD (Fig. S4c). The rCBF did not significantly increase (Fig. S4d).

Correlations between changes in rCBF and in MMSE and ADAS-Jcog scores in responders after 6 months of donepezil therapy

Table 4 shows that the rCBF in bilateral PCC (BA31) significantly correlated with MMSE and ADAS-Jcog scores ($P < 0.01$). The rCBF in the left PCC (BA23) and right ACC significantly correlated with MMSE scores, and those in the left PCC (BA23) and bilateral ACC correlated with ADAS-Jcog scores ($P < 0.05$).

Discussion

The present SPECT follow-up study successfully showed that rCBF increased transiently in the PCC of patients with AD who had relatively high baseline MMSE scores and cognitive improvement (responders). Statistical maps between baseline and 6 months showed obvious rCBF restoration mainly in the bilateral rostral part of the PCC, which
correlated with improvements in cognitive function. Statistical maps of responders compared with normal individuals showed a typical decrease in the rCBF associated with AD at baseline and at 18 months. These findings showed that rCBF can be restored in the PCC, and that it is associated with transient cognitive improvement.

The findings from the present study do not necessarily contradict those of previous studies (Table 1). Presumable causes of differences between the previous and the present findings are baseline MMSE, subgroup analysis, interval to follow-up imaging, tracers, and imaging analysis. In particular, we considered that three conditions of high vulnerability of the PCC rCBF, because of the vulnerability of the 

cerebral cortex; Rt., right.

The rCBF in the PCC has not previously been measured in studies using ROI analyses, because the PCC was included in a larger ROI along with other regions. For example, the pericallosal region on a 3D stereotaxic ROI template includes the upper precuneus, ACC and the PCC. ROI should be placed on PCC properly.

Changes in MMSE and ADAS-Jcog scores significantly correlated with rCBF changes in the PCC. Previous studies have found that changes in MMSE scores and rCBF correlate in a large left fronto-temporal region, the left frontal lobe and limbic lobe, as well as in the parietal and temporal segment. Among these three studies, the patients in a study by Shimizu et al. had mild AD, which was essentially identical to the patients described herein, and

| Table 4 Correlation coefficients (r) between regional cerebral blood flow changes and changes in Mini-Mental State Examination and Japanese version of the Alzheimer’s disease assessment scale-cognitive scale scores from baseline to 6 months later in responders |
|----------------|---------|----------------|
|                | MMSE   | ADAS-Jcog      |
| PCC (BA23)     |         |                |
| Rt.            | 0.293  | -0.387         |
| Lt.            | 0.469* | -0.511*        |
| PCC (BA31)     |         |                |
| Rt.            | 0.690† | -0.665†        |
| Lt.            | 0.701† | -0.662†        |
| Precuneus      |         |                |
| Rt.            | 0.418  | -0.423         |
| Lt.            | 0.301  | -0.375         |
| ACC (BA24)     |         |                |
| Rt.            | 0.509* | -0.495*        |
| Lt.            | 0.411  | -0.496*        |

*P < 0.05; †P < 0.01.ACC, anterior cingulate cortex; ADAS-Jcog, Japanese version of the Alzheimer’s disease assessment scale-cognitive scale; BA, Brodmann’s area; Lt., left; MMSE, Mini-Mental State Examination; PCC, posterior cingulate cortex; Rt., right.
their MMSE scores increased after therapy.\textsuperscript{10} The ROI of the limbic lobe in their study included the PCC, which might have contributed to the positive correlation between the changes in MMSE scores and limbic ROI. In contrast, the patients in the other two studies had more advanced AD, and their MMSE scores declined with therapy.\textsuperscript{9,14} The PCC in the patients in these studies might have become unresponsive to donepezil, which led to the subsequent disappearance of the correlation with MMSE scores. Thus, brain regions where changes in rCBF and MMSE correlated might vary according to clinical stage.

Changes in the PCC rCBF could be associated with connective changes in the region, considering the abundance of connections between PCC and the whole brain. The mechanisms of the reduced rCBF and metabolism in the PCC of patients with AD have been attributed to disconnection rather than focal volume loss.\textsuperscript{28} Our finding that donepezil evoked restoration of rCBF in the PCC of responders might have relevance to restored connectivity to cholinergic nucleus.

A limitation of the present study was that we could not clarify causes of the difference between responders and non-responders. Although baseline MMSE and years of education did not significantly differ between the two groups, non-responders might have decreased cognitive reserve, considering baseline parietal non-significant hypoperfusion (Fig. S4). The apolipoprotein Eε4 allele that might have promoted the progress of AD was not examined in the present study. Furthermore, the environment\textsuperscript{29} and lifestyle\textsuperscript{30} factors might also have affected the response.

In conclusion, brain perfusion SPECT identified increased rCBF in the PCC after 6 months of donepezil therapy. This increase was evident in patients with responders and high baseline MMSE. The restoration of PCC rCBF correlated with cognitive function. These present findings support our hypothesis that PCC hypoperfusion reflects integration of cholinergic dysfunction.

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Disclosure statement

The authors declare no conflict of interest.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher’s web site:

Figure S1 Image maps of stereotactic extraction estimation on the medial surface. (a) BA23 (orange), (b) BA31 (red), (c) precuneus (red) and (d) BA24 (yellow).

Figure S2 Change of Mini-Mental State Examination and Alzheimer’s disease assessment scale-cognitive scale scores in responders and non-responders. *P < 0.05 and †P < 0.05 denotes significant difference compared with non-responders and baseline, respectively (Tukey–Kramer multiple comparison test).

Figure S3 Statistical maps of baseline regional cerebral blood flow (rCBF) difference between responders and non-responders. (a) Relative difference in rCBF between responders and non-responders (two-sample t-test). The rCBF in the blue (decreasing) regions of non-responders is smaller than that of responders. (b) Statistical map of relative increase in rCBF with cut-off Z-score of 1.96 (corresponding to P ≤ 0.05; two-tailed test). There were no regions that showed a significant difference between the two cohorts.

Figure S4 Statistical maps of regional cerebral blood flow (rCBF) changes in responders and non-responders. (a) Relative change in rCBF of responders from baseline to 6 months (two-sample t-test). (b) Statistical map of relative increase in rCBF of responders with cut-off Z-score of 1.96 (corresponding to P ≤ 0.05; two-tailed test). The rCBF in the bilateral posterior cingulate cortex was increased significantly. (c) Relative change in rCBF of non-responders from baseline to 6 months (two-sample t-test). (d) Statistical maps of relative increase in rCBF of non-responders with cut-off Z-score of 1.96.