Molecular Imaging of Acetylcholine Esterase Using [^{11}C]Donepezil: Application to Alzheimer's Disease

Kazuhiko Yanai*[1,2)], Manabu Tashiro, and Nobuyuki Okamura

* Professor
Department of Pharmacology, Graduate School of Medicine and
Cyclotron and Radioisotope Center
E-mail: yanai@mail.cc.tohoku.ac.jp

Abstract

Donepezil hydrochloride is widely used for the treatment of Alzheimer's disease (AD). However, the biodistribution of donepezil in the brain after administration is not precisely understood. To visualize in vivo binding of donepezil to acetylcholinesterase (AChE) in the brain and to establish a method for determining the optimal dose of donepezil in AD patients. [5-^{11}C-methoxy]donepezil ([^{11}C]donepezil) was radiolabeled as a positron emission tomography (PET) tracer. The biodistribution of [^{11}C]donepezil was measured by PET in 10 AD patients and 6 aged normal subjects. Two AD patients underwent additional PET measurements after oral administration of donepezil for 6 months. [^{11}C]donepezil-PET images demonstrated high densities of tracer distribution in AChE-rich brain regions such as the striatum, thalamus, and cerebellum. Patients with mild AD exhibited about 18-20% reduction of donepezil binding in neocortex and hippocampus, while patients with moderate AD exhibited about 24-30% reduction of donepezil binding throughout the brain. Orally administered donepezil (5 mg per day) induced 61.6 – 63.3% reduction of donepezil binding in AD brains. The distribution volume of [^{11}C]donepezil in hippocampus was significantly correlated with MMSE scores in AD patients. [^{11}C]donepezil-PET enables quantitative measurement of donepezil binding in the brain. AD patients exhibited reduction of donepezil binding in the brain, even in the early stage of disease. Longitudinal evaluation by this technique enables determination of AChE binding occupancy by orally administered donepezil. This technique will provide a new surrogate marker for evaluation and prediction of response to donepezil treatment.

1. Introduction

Cholinergic deficit is consistently found in the brain of patients with Alzheimer's disease (AD). Reduction in the activity of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) is evident in AD brains and correlated with cognitive decline. For this reason, cholinergic enhancement is a major approach to the treatment of AD. Several attempts have been made to treat AD using cholinergic receptor agonists or AChE inhibitors (AChEI). The latter have been successfully translated into clinically applicable means of treatment. Currently, several AChE inhibitors are widely prescribed to improve cognitive function in patients with AD. However, not all patients respond to these treatments. It is thus important to identify factors that determine individual responses to treatment with AChE inhibitors.

Functional imaging of cholinergic function is a useful strategy for determination of the clinical status of demented patients. Currently, in vivo monitoring of brain AChE activity using positron emission tomography (PET) is beneficial in developing strategies for dementia therapy. [^{11}C]MP4A and [^{11}C]PMP, which are metabolically trapped acetylcholine analogues, have been successfully applied to the evaluation of AChE activity in the brain. PET studies in AD patients have demonstrated reduction of AChE activity in the early stage of disease, with degree of reduction correlated with that of cognitive dysfunction. Another strategy involves use of AChEIs themselves as radiotracers. This method enables direct investigation of the pharmacokinetics of AChEIs. [^{11}C]physostigmine, [^{11}C]methyl-tacrine, and [^{11}C]CP-126,998 have been designed as radiotracers for clinical PET study. In vivo imaging technique using such radiotracers can measure the concentrations of tracer-binding AChE. If these radiotracers and therapeutic drugs competitively bind to AChE, the occupancy of binding sites on AChE by therapeutic drugs could be measured by subtraction from pre-treatment of post-treatment PET scans.

Donepezil hydrochloride is currently the AChEI most widely used for the treatment of AD. It exhibits selective binding of AChE compared with butyrylcholinesterase (BuChE). Radiolabeled donepezil can thus be used as a tracer to measure brain concentrations of AChE. If the distribution of donepezil in the brain can be quantitatively measured by PET, this will be useful for pharmacological evaluation of AChEIs and for prediction of efficacy of treatment with donepezil. In this study, we performed PET examinations using [5-^{11}C-methoxy]donepezil ([^{11}C]donepezil) and determined the in vivo binding characteristics of donepezil in AD patients.

2. Methods

2.1. Subjects and patients

Six aged normal subjects and 10 patients with probable AD were studied to examine the distribution of [^{11}C]donepezil in the brain. The AD patients were recruited through The Tohoku University Hospital Dementia Patients Registry. The diagnosis of AD was made according to the National Institute of Neurologic Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria. AD patients were further divided into two groups by severity: a mild AD group (n = 5; MMSE score ≥23 points) and a moderate AD group (n = 5; MMSE score <20 points). The normal control group was comprised of volunteers without impairment of cognitive function who had no cerebrovascular lesions on magnetic resonance (MR) images. After complete description of the study to the patients and subjects, written informed consent was obtained from them. PET study was performed within 3 months after the completion of medical and neuropsychological examination. Although no significant difference in age was observed between the mild AD group and aged normal group, the moderate AD group was older than the aged normal group (Table 1). The MMSE score of the aged normal subjects (Mean ± SD 29.7 ± 0.8) was significantly higher than that of the mild AD (25.0 ± 1.6) and moderate AD (15.4 ± 2.7) subjects.

Table 1. Subjects and patients demographics

		Gender	Age	MMSE
Aged normal	AN1	M	64	30
	AN2	M	61	30
	AN3	F	59	30
	AN4	F	60	30
	AN5	M	74	28
	AN6	F	75	30
	Mean		65.5	29.7
	s.d.		7.2	0.8
Mild AD	AD1	F	77	24
	AD2	F	72	23
	AD3	M	71	26
	AD4	F	66	25
	AD5	M	69	27
	Mean		71.0	25.0
	s.d.		4.1	1.6
Moderate AD	AD6	F	77	14
	AD7	F	78	12
	AD8	F	79	19
	AD9	M	84	17
	AD10	F	81	15
	Mean		79.8	15.4
	s.d.		2.8	2.7

2.2. Radiosynthesis of [5-^{11}C-methoxy]donepezil

Synthesis of [^{11}C]donepezil was performed (Fig. 1) as described previously. Briefly, 5'-O-desmethylprecursor (M2) was dissolved in methylethylketone and then tetrabutylammonium hydroxide was added. [^{11}C]Methyl iodide was prepared from [^{11}C]CO2 and converted to [^{11}C]methyl triflate ([^{11}C]MeOTf). [^{11}C]Donepezil was produced on the loop from [^{11}C]MeOTf and purified by high performance liquid chromatography (HPLC). The radioactivity obtained was 155.4-814 MBq (4.2-22 mCi), and radiochemical yield was 25-30% based on [^{11}C]MeOTf after decay-correction. Specific activity was 111-354 GBq/μmol at the end of synthesis (30-40 min from the end of 11C production). Radiochemical purity was greater than 99%.

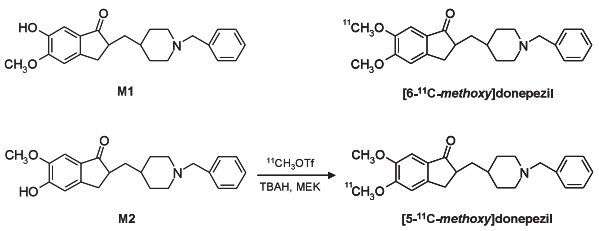

Fig. 1. Chemical structures and radiosynthesis of donepezil and its metabolites.

2.3. PET acquisition protocols

The protocol of the PET study was approved by the Committee on Clinical Investigation at The Tohoku University School of Medicine and the Advisory Committee on Radioactive Substances at Tohoku University. The [^{11}C]Donepezil PET study was performed using a SET-2400W PET scanner (Shimadzu Inc., Japan) under resting condition with eyes closed. Following a ^{68}Ge/Ga transmission scan of 7 min duration, an emission scan was started soon after intravenous injection of 7.1 - 9.5 mCi of [^{11}C]donepezil. Emission data were acquired for 60 min. Standardized uptake value (SUV) images were obtained by normalizing tissue concentration by injected dose and body mass. Arterialized venous blood samples were obtained from a hand vein, heated in a far-infrared mat, and radioactivity was measured in a well-type scintillation counter. Sampled plasma (2 ml) was denaturated with 1M HClO4: MeCN (7:3) and centrifuged at 3,000 x G for 3 min. The supernatant solution was injected into a column (YMC ODS A-324, YMC Co., Ltd., Kyoto, Japan; 10 mm i.d. x 30 cm long) with a solvent system of 0.1M ammonium formate-acetonitrile (60/40) at a flow rate of 5.0 ml/min. The elutes were collected at time intervals of 0.5 min and were counted for radioactivity with a gamma counter.

2.4. Image analysis

Region of interest (ROI) analysis was performed to evaluate the regional distribution of [^{11}C]donepezil. Circular ROIs (1.0 cm in diameter) were placed on individual axial PET images in the cerebellar hemisphere, striatum, thalamus, lateral frontal cortex [Brodmann's areas (BA) 44, 45, 46, and 47], lateral temporal cortex (BA 20, 21, and 22), parietal cortex (BA 39 and 40), occipital cortex (BA 17), anterior cingulate cortex (BA 24 and 32), posterior cingulate cortex (BA 23 and 31), and medial temporal cortex (BA 27, 28, 34, and 35), referring to the individual MR images. The distribution volume (DV) of [^{11}C]donepezil was calculated by Logan's graphical analysis13, since donepezil reversibly binds AChE. Using this method, the DV in each ROI was determined from the slopes obtained from the values of each ROI and input function from metabolite-corrected plasma radioactivity. The slopes were determined from the last 15 points of the respective regions. Details of quantitative analysis will be described elsewhere.

2.5. Statistical analysis

Differences in age, MMSE score, and DV among the three groups were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test (GraphPad Prism Software). For each analysis, findings were considered significant at $p<0.05$.

3. Results

Tissue time activity curves (TAC) of [^{11}C]donepezil in the brain indicated initial rapid uptake of radioactivity followed by gradual clearance from the brain in both aged normal (Fig. 2A) and AD subjects (Fig. 2B). Relatively high concentrations of radioactivity of [^{11}C]donepezil were observed in AChE-rich brain regions such as the striatum, thalamus, and cerebellum, whereas radioactivity uptake in the neocortex including frontal, temporal, and parietal cortices was moderate. Plasma radioactivity of [^{11}C]donepezil peaked at 30-60 s post-injection, followed by a rapid decline.

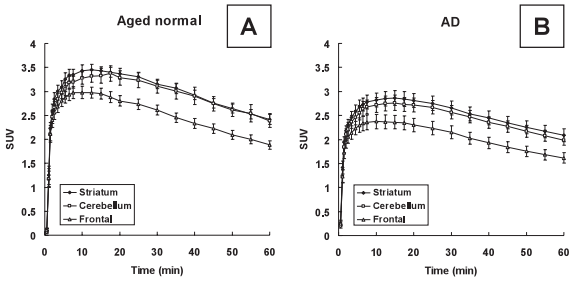

Fig. 2. Time activity data for [^{11}C]donepezil PET in humans. Brain SUV time activity curves for aged normal subjects (A) and AD patients (B) are shown. The dotted line indicates metabolite-corrected time activity curve.

Proportions of unchanged [^{11}C]donepezil in plasma were 91.0 ± 7.0 %, 88.1 ± 12.5 %, and 82.5 ± 5.1 % at 5, 15, and 30 min post-injection, respectively. The metabolite-corrected plasma time–activity curve was used to calculate specific DVs from the region-of-interest–derived regional time–activity curve. [^{11}C]donepezil exhibited linear regression curves on Logan plot analysis in all brain regions examined (Fig. 3).

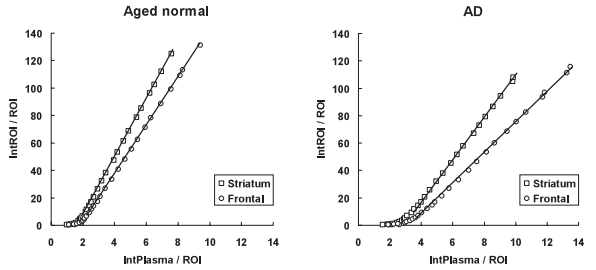

Fig. 3. Logan plots for the striatum (open squares) and frontal cortex (open circles) for aged normal subjects (A) and AD patients (B)

Since the slopes of regression lines represent the DV of the tracer, these findings indicate higher DV of donepezil in the striatum than in frontal cortex. Parametric images of [^{11}C]donepezil DV clearly revealed higher concentrations of tracer distribution in the striatum and cerebellum than in neocortex. Patients with mild AD exhibited reduction of DV in the hippocampus and neocortex, compared with aged normal subjects. The magnitude of DV reduction in the mild AD group was about 20% in the hippocampus and 18% in temporal and parietal cortices. In patients with moderate AD, DV reduction was evident throughout the brain (Fig. 4 and 5, Table 2). The magnitude of DV

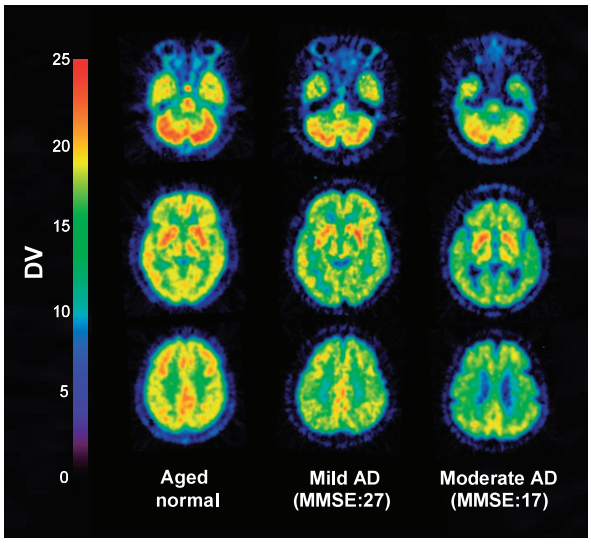

Fig. 4. Distribution volume images of [11C]donepezil in aged normal subjects (left), patients with mild AD (middle), and patients with moderate AD (right).

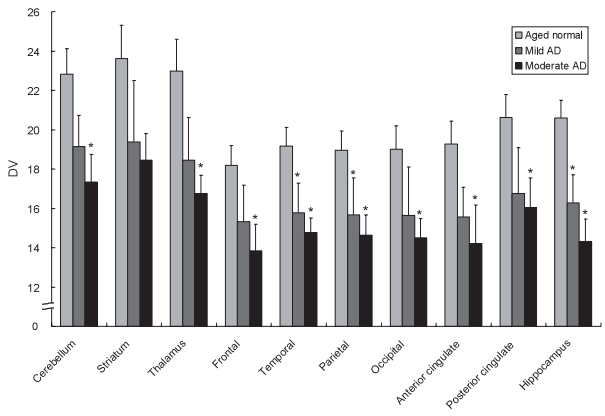

Fig. 5. Regional distribution volume data in aged normal subjects, mild AD, and moderate AD patients.

reduction was about 30% in the hippocampus and 24% in frontal, temporal, and parietal cortices. Two AD patients (AD3 and AD10) underwent another PET scan after treatment with 5 mg of donepezil for 6 months. Orally administered donepezil induced substantial reduction of DV in all regions of brain examined (Fig. 6). Mean DV reduction was 61.6 % (range 55.5 - 65.5%) in patient 1 (AD3) and 63.3% (range 54.2 - 78.8%) in patient 2 (AD10). Finally, the correlation of donepezil binding with severity of dementia was examined within the AD patient group. As shown in Fig. 7, DV value in the hippocampus was significantly correlated with MMSE scores of AD patients.

4. Discussion

This PET study demonstrated that intravenously administered [^{11}C]donepezil rapidly enters the brain and is mainly distributed in the striatum, thalamus, and cerebellum, which are known to contain high densities of AChE compared to cerebral cortex and hippocampus. This finding is consistent with the findings of our previous study in rats. The regional distribution of [^{11}C]donepezil was also consistent with regional AChE activity determined in a human postmortem study, suggesting selective binding of donepezil to AChE.

Patients with moderate AD exhibited significant reduction of [^{11}C]donepezil DV in all brain regions examined. Furthermore, temporo-parietal and hippocampal DVs were significantly reduced even in patients with mild AD. These reductions suggest early involvement of the cholinergic system in AD, since the AChE in brain is predominantly located in presynaptic cholinergic neurons. A previous [^{11}C]MP4A PET study demonstrated 21% reduction of hippocampal AChE activity in patients with early-onset AD. We observed an approximately 20% reduction in hippocampal DV in the mild AD group and 30% reduction in the moderate AD group. These findings suggest that the concentration of donepezil-binding AChE is matched by regional AChE activity. In postmortem study, AD patients exhibit reduction of AChE activity, and this reduction is correlated with severity of dementia. We observed that DV in the hippocampus was correlated with cognitive status in AD patients, a finding in accord with postmortem data. However, it is important to note that partial volume effect might have influenced regional DV reduction in the hippocampus. Analysis after partial volume correction is therefore needed to further establish the relationship between regional DV of [^{11}C]donepezil and severity of dementia in AD.

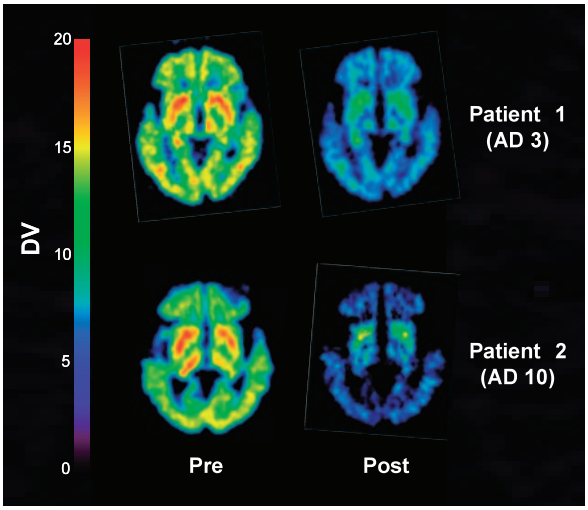

Fig. 6. Distribution volume images before and after oral administration of donepezil in AD patients.

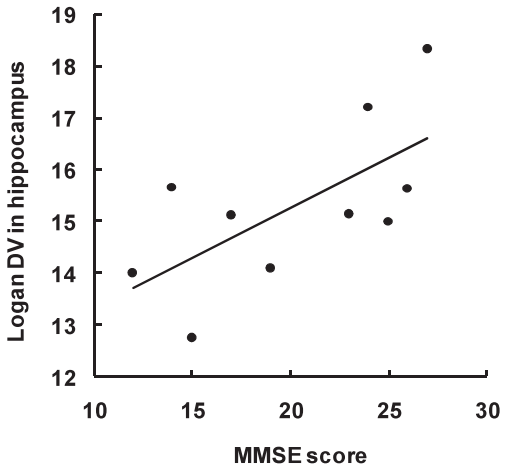

Fig. 7. Correlation between MMSE scores and distribution volume in the hippocampus of AD patients.

Compared with previously reported findings of PET imaging with [^{11}C]MP4A6 and [^{11}C]PMP7, the present [^{11}C]donepezil-PET study demonstrated relatively higher cortical retention of radiotracer, suggesting the existence of alternative binding sites for donepezil other than AChE. Donepezil is reported to have high binding affinity for σ1-receptors, which are widely distributed in the brain including cerebral cortex, hippocampus, and cerebellum. A recent human PET

study using σ1-receptor-specific radioligand demonstrated prominent reduction of σ1-receptor density in the cerebral cortex and cerebellum of AD patients. Thus, concomitant binding of donepezil to σ1-receptors might have contributed to the distinctive distribution of [^{11}C]donepezil we observed in the brain.

Previously, the tracer kinetics of [^{11}C]donepezil with labeling of the methoxy group at position 6 ([6-^{11}C-methoxy]donepezil) were examined, to test this agent as a candidate for a PET radioligand. However, the regional brain distribution of this radiotracer did not reflect the distribution of AChE in the brain. In contrast, our previous study yielded successful in vivo visualization of AChE by donepezil labeled with ^{11}C at the methoxy group at position 5 ([5-^{11}C-methoxy]donepezil). The differences between these findings might be attributable to the affinity of unlabeled metabolites to AChE. Indeed, the unlabeled metabolite of [6-^{11}C-methoxy]donepezil (M1 in Fig. 1) has high binding affinity for AChE (IC50 = 6.4 nM), resulting in competition for binding between ^{11}C-labeled tracer and unlabeled metabolite, while the metabolite of [5-^{11}C-methoxy]donepezil (M2 in Fig. 1) exhibits lower affinity of binding to AChE (IC50 = 1.1 μM) than M1. [5-^{11}C-methoxy]donepezil is thus suitable for detection of AChE in vivo. In addition, the specific radioactivity of [5-^{11}C-methoxy]donepezil in this study (111-354 GBq/μmol) was higher than that of [6-^{11}C-methoxy]donepezil in the previous study. High specific activity of [^{11}C]donepezil might therefore be another contributing factor of successful visualization of AChE.

Post-treatment evaluation following administration of 5 mg donepezil per day revealed remarkable reduction (61.6-63.3 % compared to pre-treatment scan) of [^{11}C]donepezil binding throughout the brain. This indicates that the AChE occupancy by donepezil, when administered in daily doses of 5 mg, was about 35-40 % in these two patients. A previous PET study using [^{11}C]MP4A revealed a mean 39% reduction in AChE activity after oral administration of 3 to 5 mg donepezil. Intravenous administration of donepezil in monkeys also resulted in a mean 27% reduction of AChE activity at a dose of 100 μg/kg. These findings together suggest that inhibition of AChE activity matches occupancy of AChEI binding sites. Moreover, orally administered donepezil (5 mg) induced substantial inhibition (43 - 62%) of the binding of another radiotracer, [^{11}C]CP-126,998, to AChE. This finding is roughly consistent with our observations. The amount of binding of orally administered donepezil to AChE is considered a key factor determining therapeutic response. AChE binding occupancy by orally administered donepezil could be modulated by blood-brain barrier permeability, tissue distribution, metabolism, and also by AChE density in the brain. In vivo evaluation of AChE occupancy could thus be a powerful strategy for determining the optimal dose of donepezil. In the future, quantitative evaluation of donepezil binding sites might be used to optimize regimens of treatment with donepezil and to predict the response to treatment.

In conclusion, in this study the distribution of donepezil in human brain was successfully visualized using [^{11}C]donepezil and PET. Graphical analysis by Logan plots can be used to obtain quantitative estimates of specific donepezil binding. AD patients exhibited significant reduction of donepezil distribution, even in the early stage of disease. This imaging technique will be useful as a new surrogate marker for evaluation of treatment with donepezil.

Acknowledgements

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