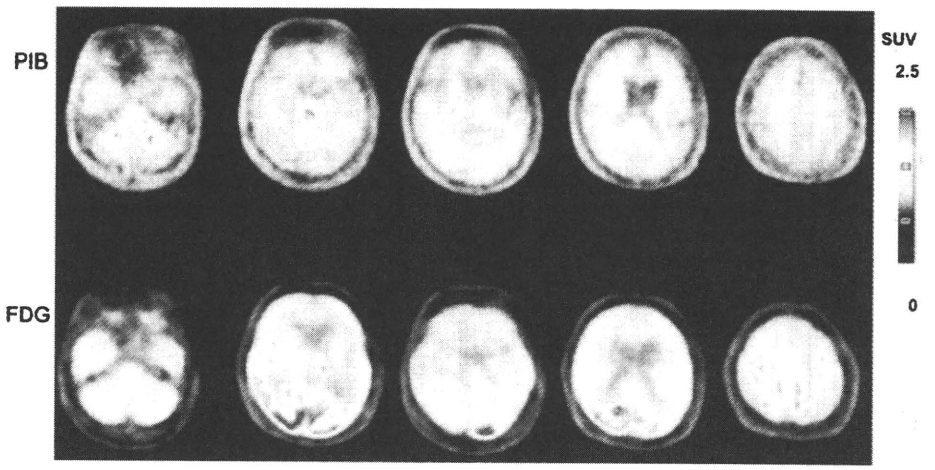

Fig. 2 *Upper panel* PIB-PET shows no accumulation of amyloids in the cortex. *Lower panel* FDG-PET shows decreased glucose metabolism in the frontal and temporal lobes with left-side dominancy associated with decreased metabolism in the right cerebellar hemisphere

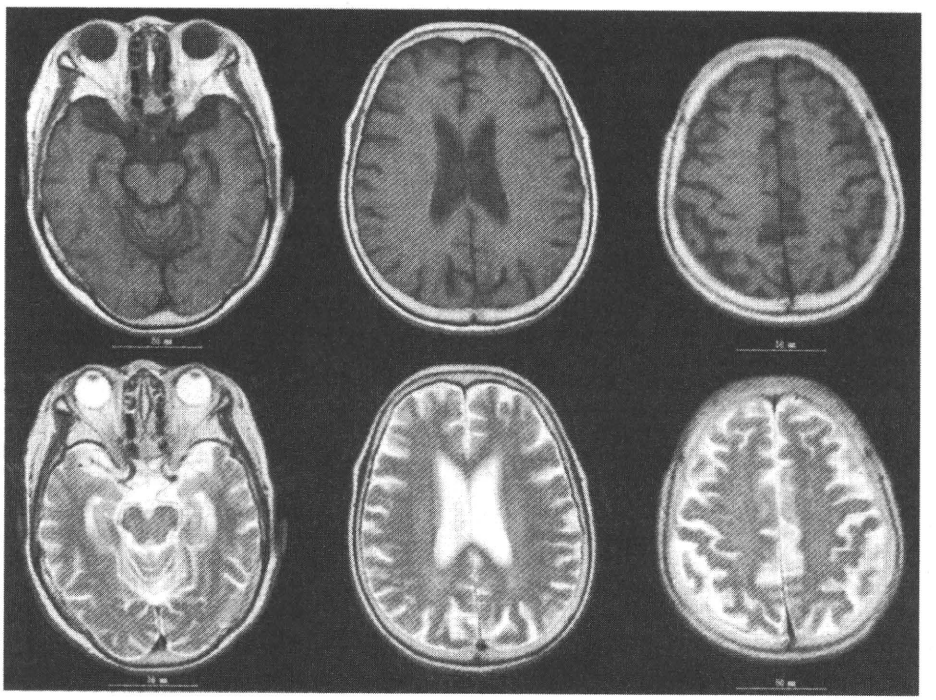

Fig. 3 Atrophy of bilateral medial temporal areas. Age-associated diffuse atrophy of the cerebral cortex

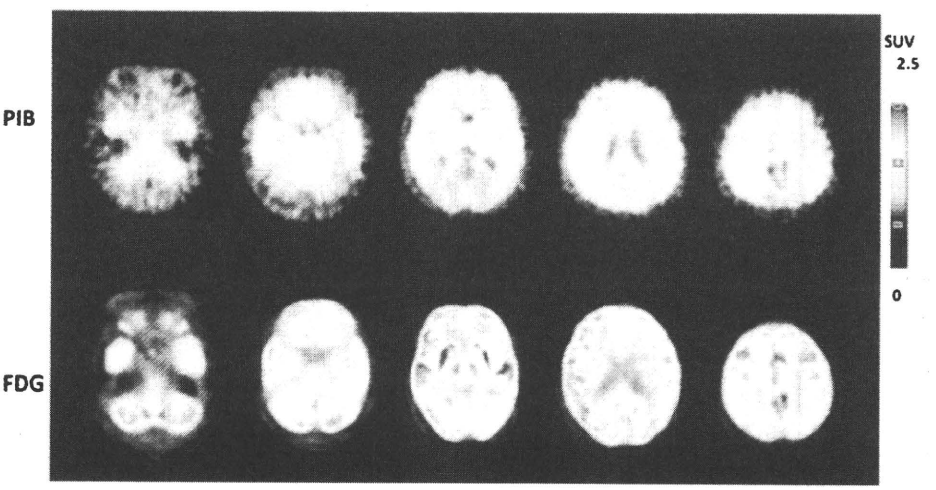

Fig. 4 *Upper panel* PIB-PET shows accumulation of amyloids mainly in the frontal lobe, anterior and posterior cingulate gyrus, precuneus, and also in the parietal lobe and lateral temporal lobe. *Lower panel* FDG-PET shows decreased glucose metabolism in bilateral parietal lobes with left-side dominancy and left lateral temporal lobe [15–23]

Table.1 Previous and present cases of motor neuron disease associated with dementia

	Tsuchiya et al. [15] 1	Ishihara K et al. [16]	Tsuchiya et al. [17] 2	Yokota O et al. [18]	Yamamoto et al. [19]	Yamamoto et al. [19]	Matsuda et al. [20]	Osoegawa et al. [21]	Yamashita et al. [22]	Rusina et al. [23]	Rusina et al. [23]	Present case 1	Present case 2
Clinical features													
Age of onset (years)	69	52	30	48	51	64	65	56	72	68	62	61	79
Age of emergence of dementia (years)	70	52	30	48	51	64	67	58	73	69	62	61	79
Age of emergence of motor neuron disease (years)	69	52	44	53	54	64	67	56	72	68	62	61	81
Duration (years)	2	7	15	6	4	4	3	4	1.5	20 months	2		
Sex	Female	Female	Female	Female	Male	Female	Male	Male	Female	Female	Male	Male	Female
Initial symptoms	Dysarthria and gait disturbance	Speech difficulties	Abnormal behavior	Abnormal behavior	Personality change	Personality change	Motor aphasia	Muscle weakness	Bulbar palsy	Bulbar palsy and motor impairment	Bulbar palsy and cognitive impairment	Cognitive impairment, muscle atrophy, and muscle weakness	Cognitive impairment
Prominent symptoms	Bulbar palsy and gait disturbance	Bulbar palsy	Bulbar palsy	Bulbar palsy and gait disturbance	Bulbar palsy	Bulbar palsy and gait disturbance	Bulbar palsy	Muscle atrophy and muscle weakness	Bulbar palsy	Muscle weakness	Muscle weakness	Muscle atrophy and muscle weakness	Bulbar palsy
Upper motor neuron signs	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+)	(+)	(+)	(+)	(+)
Family history	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	Unknown	(−)	(−)	(−)	(−)
Part of brain atrophy													
Frontal lobe	(−)	(−)	(+)	Unknown	Bilateral	Bilateral	Bilateral					Bilateral	(−)
Temporal lobe	Right	(−)	(+)	Unknown	Bilateral	Right	Bilateral					(−)	(−)
Caudate nucleus	(−)	(+)	(+)	Unknown	(−)	(−)	(−)					(−)	(−)
Hippocampus	(−)	(−)	(−)	(−)	(−)	(−)	(−)					Left	(+)
Histological features													
Tau pathology	(−)	(−)	(−)	Neurofibrillary tangles in the frontal and temporal lobe	(−)	(−)							
Ubiquitin-positive inclusions	(+)	(−)	(+)	(−)	(+)	(+)							
Diagnosis	FTD-MND	FTLD associated with MND	†FTD-MND	‡FTLD associated with §MND	FTD-MND	FTD-MND	FTD-MND	Alzheimer's disease with FTD-MND	Alzheimer's disease associated with MND	Alzheimer's disease associated with MND	Alzheimer's disease associated with MND	FTD-MND	Alzheimer's disease associated with MND

† *FTD-MND* frontotemporal dementia with motor neuron disease, ‡ *FTLD* frontotemporal lobar degeneration, § *MND* motor neuron disease

and posterior cingulate gyrus, precuneus, and also in the parietal and lateral temporal lobes. FDG-PET indicated depressed metabolism of glucose in both the parietal lobes and in the left lateral temporal lobe (Fig. 4). These findings suggested that the patient had AD since 2005, and had slowly progressive MND since 2007.

Discussion

The novel PET tracer ^{11}C-PIB has a high affinity for fibrillar amyloid beta protein (Aβ). Klunk W et al. [4] reported that the in vitro 2-(4′-methylaminophenyl) benzothiazole (BTA-1) binding was over tenfold higher in the AD brain than in the normal brain, and that the majority (94%) of the binding was specific for amyloid, and high-affinity BTA-1 was observed only in the AD brain gray matter. However, Aβ accumulation is one of the pathologic hallmarks of AD, but not of frontotemporal lobar degeneration (FTLD), as shown in the criteria proposed by McKhann et al. [1] in 2001; according to the criteria, FTLD is classified into three major groups depending on the presence or the absence of tauopathy and ubiquitinopathy. Alternatively, according to the criteria proposed by Cairns et al. [5] in 2007, FTLD is classified in terms of the presence or the absence of the 43-kDa transactive response (TAR) DNA-binding protein (TDP-43 or TARDBP), which was identified by Arai T et al. [6]. ^{11}C-PIB binds specifically to fibrillar Aβ in AD brains, but shows a low binding affinity to brains from patients with non-Aβ dementias, including FTLD. PIB-PET demonstrated significantly higher ^{11}C-PIB retention in the gray matter of AD patients than that of FTLD patient [7]. In a previous study conducted on 30 ALS patients, 50% had Aβ plaques at histopathological examination; however, of the seven cases without cortical motor neuron inclusions, only two had neuritic plaques [8].

Table 1 summarizes previous and present cases of MND associated with dementia. In 7 of the 8 cases of FTLD associated with MND, including FTD-MND, the age of onset ranged from the half of the fifth to the half of the sixth decade of life, as in our case 1. The mean age of onset of FTLD with MND was 55.6 $\pm$ 15.9 years, whereas that associated with MND varied around 50 years. About the cognitive features, most patients with FTD/ALS show almost the same cognitive and behavioural impairments of FTD patients.

There are some cases where the clinical course of FTD is similar to that of AD, and vice versa; hence, clinical course is not helpful in confirming the diagnosis. Reñé et al. [9] reported that MRI showed frontal and/or temporal atrophy in 62% of the FTLD cases, and single-photon emission computed tomography (SPECT) showed frontal and/or temporal hypoperfusion in 75% of the FTLD cases. It has been reported that FDG-PET is useful in the differential diagnosis of AD from FTLD with more than 85% sensitivity and specificity [10]. Recently, Zhou [11] reported the efficacy of the differential diagnosis of AD from variant form of FTD with the resting state functional magnetic response imaging (RS-fMRI). On the other hand, the accumulation of amyloids is observed in AD but not in FTLD. Our study showed that AD associated with ALS showed positive PIB scans, whereas FTD-MND showed negative scans. In some cases, neither the clinical course nor radiological analyses other than functional neuroimaging techniques are useful in discriminating AD from FTD, especially in the initial stage of the disease.

Recently, the TDP-43 protein has been identified as the cause of FTD/ALS [6] and the mutation of SOD1 gene has been already reported as the cause of familial ALS [12]. Some mutations of the TDP-43 gene may contribute significantly to the aggregation and forming amyloid structures inducted by the C-terminal fragments of the TDP-43 [13]. On the other hand, the SOD1 mutant increased aggregation propensity and formation of amyloid like fibrils [14]. Because these studies suggest that their mutation affect the amyloid formation in the brain of the FTLD patients, we have to consider the possibilities of these mutations affect to our PET data. In the future, we would like to analyze the presence of these mutations of our patients' gene.

Our study suggests that PIB-PET can be considered as a useful tool to discriminate the different proteinopathies that cause neurodegenerative diseases, as dementia associated with ALS.

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Cholinergic imaging in corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia

Shigeki Hirano,[1,2] Hitoshi Shinotoh,[1,3] Hitoshi Shimada,[1,4] Akiyo Aotsuka,[5] Noriko Tanaka,[1,6] Tsuneyoshi Ota,[1,7] Koichi Sato,[1,8] Hiroshi Ito,[1] Satoshi Kuwabara,[9] Kiyoshi Fukushi,[10] Toshiaki Irie[10] and Tetsuya Suhara[1]

1 Molecular Neuroimaging Group, Molecular Imaging Centre, National Institute of Radiological Sciences, Chiba 263-8555, Japan
2 Department of Neurology, JR Tokyo General Hospital, Tokyo 151-8528, Japan
3 Asahi Hospital for Neurological Diseases, Chiba, 270-0022, Japan
4 Section for Human Neurophysiology, Research Centre for Frontier Medical Engineering, Chiba University, Chiba 263-8522, Japan
5 Department of Neurology, Chiba Aoba Municipal Hospital, Chiba 260-0852, Japan
6 Department of Neurosurgery, Tokyo Women's Medical University, Medical Centre East, Tokyo 116-8567, Japan
7 Department of Psychiatry, Juntendo University School of Medicine, Tokyo 113-8431, Japan
8 Department of Psychiatry, Teikyo University Chiba Medical Centre, Chiba 299-0111, Japan
9 Department of Neurology, Chiba University School of Medicine, Chiba 260-8677, Japan
10 Molecular Probe Group, Molecular Imaging Centre, National Institute of Radiological Sciences, Chiba 263-8555, Japan

Correspondence to: Hitoshi Shinotoh,
Molecular Neuroimaging Group,
Molecular Imaging Centre,
National Institute of Radiological Sciences,
4-9-1 Anagawa, Inage-ku, Chiba-shi,
Chiba, 263-8555, Japan
E-mail: hitoshi.shinoto@nifty.com

Corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia are all part of a disease spectrum that includes common cognitive impairment and movement disorders. The aim of this study was to characterize brain cholinergic deficits in these disorders. We measured brain acetylcholinesterase activity by [^{11}C] *N*-methylpiperidin-4-yl acetate and positron emission tomography in seven patients with corticobasal syndrome (67.6 ± 5.9 years), 12 with progressive supranuclear palsy (68.5 ± 4.1 years), eight with frontotemporal dementia (59.8 ± 6.9 years) and 16 healthy controls (61.2 ± 8.5 years). Two-tissue compartment three-parameter model and non-linear least squares analysis with arterial input function were performed. k_3 value, an index of acetylcholinesterase activity, was calculated voxel-by-voxel in the brain of each subject. The k_3 images in each disease group were compared with the control group by using Statistical Parametric Mapping 2. Volume of interest analysis was performed on spatially normalized k_3 images. The corticobasal syndrome group showed decreased acetylcholinesterase activity (k_3 values) in the paracentral region, frontal, parietal and occipital cortices ($P < 0.05$, cluster corrected). The group with progressive supranuclear palsy had reduced acetylcholinesterase activity in the paracentral region and thalamus ($P < 0.05$, cluster corrected). The frontotemporal dementia group showed no significant differences in acetylcholinesterase activity. Volume of interest analysis showed mean cortical acetylcholinesterase activity to be reduced by 17.5% in corticobasal syndrome ($P < 0.001$), 9.4% in progressive supranuclear palsy ($P < 0.05$) and 4.4% in frontotemporal dementia (non-significant), when compared with the control group. Thalamic acetylcholinesterase activity was reduced by 6.4% in corticobasal syndrome

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(non-significant), 24.0% in progressive supranuclear palsy ($P<0.03$) and increased by 3.3% in frontotemporal dementia (non-significant). Both corticobasal syndrome and progressive supranuclear palsy showed brain cholinergic deficits, but their distribution differed somewhat. Significant brain cholinergic deficits were not seen in frontotemporal dementia, which may explain the unresponsiveness of this condition to cholinergic modulation therapy.

Keywords: corticobasal syndrome; progressive supranuclear palsy; frontotemporal dementia; acetylcholinesterase; positron emission tomography

Abbreviations: MMSE = Mini-Mental State Examination; MP4A = *N*-methylpiperidin-4-yl acetate; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

It has become increasingly clear that there is a considerable overlap among corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia. Corticobasal syndrome and progressive supranuclear palsy share several clinical features such as akineto–rigid syndrome, lack of balance, falls and eye movement abnormalities (Rinne *et al.*, 1994). Non-fluent aphasia, apathy and executive dysfunction may be seen in corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia (Kertesz *et al.*, 2003; Sha *et al.*, 2006). The clinical overlap between corticobasal syndrome and progressive supranuclear palsy parallels the neuropathological overlap in these disorders (Rinne *et al.*, 1994; Litvan *et al.*, 1997; Schneider *et al.*, 1997; Bergeron *et al.*, 1998; Kertesz *et al.*, 2000). The similarities in clinical manifestations and pathological features for these neurodegenerative disorders are of interest, since they may share common underlying molecular pathomechanisms, one of which is biochemical alterations in the tau protein (Houlden *et al.*, 2001; Ingelsson *et al.*, 2007). Corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia are progressive relentless diseases with few therapeutic options. Therefore, it is important to clarify their underlying neurochemical pathologies in order to further the understanding of their pathophysiology, which may lead to some clues for effective pharmacological intervention.

There have been a few *in vivo* studies of cholinergic systems in progressive supranuclear palsy (Asahina *et al.*, 1998; Shinotoh *et al.*, 1999), but no *in vivo* studies of cholinergic systems in corticobasal syndrome and frontotemporal dementia. The aim of this study is to characterize the cholinergic deficits *in vivo* in patients with clinically diagnosed corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia. *N*-methylpiperidin-4-yl acetate (MP4A) is an acetylcholine analogue, and PET with [^{11}C]MP4A as a radiotracer allows us to measure brain acetylcholinesterase activity quantitatively *in vivo* (Irie *et al.*, 1994; Iyo *et al.*, 1997). Acetylcholinesterase is membrane-bound predominantly on presynaptic cholinergic neurons and, to a lesser degree, on postsynaptic cholinoceptive neurons in the cerebral cortex. Therefore, by measuring cortical acetylcholinesterase activity, we can assess the integrity of the ascending cholinergic system from the nucleus basalis of Meynert. In the brainstem, there are two cholinergic nuclei, pedunculopontine and laterodorsal tegmental nuclei, which send ascending cholinergic fibres mainly to the thalamus (Mesulam *et al.*, 1983). Thalamic acetylcholinesterase activity is thought to reflect the function of the ascending cholinergic systems from the pedunculopontine and laterodorsal tegmental nuclei. In the present study, we assessed the integrity of two major ascending cholinergic systems in patients with corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia by [^{11}C]MP4A positron emission tomography.

Materials and methods

Subjects

Diagnosis of corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia was based on established consensus criteria [corticobasal syndrome: (Boeve *et al.*, 2003), probable progressive supranuclear palsy: National Institute of Neurological Diseases and Stroke—the Society for progressive supranuclear palsy criteria (Litvan *et al.*, 1996), frontotemporal dementia: Lund–Manchester criteria (Neary *et al.*, 1998)]. Briefly, the clinical diagnosis of corticobasal syndrome was based on the presence of progressive asymmetric rigidity and ideomotor apraxia, as well as other findings that reflect cortical (e.g. alien limb phenomenon, cortical sensory loss, myoclonus) and extrapyramidal (e.g. bradykinesia, tremor, dystonia) dysfunction. Probable progressive supranuclear palsy was defined by gradual progressive symmetrical rigidity and bradykinesia, vertical gaze palsy and falls in the first year of disease onset. The diagnosis of frontotemporal dementia was based on (i) changes in personality and social behaviour, specifically apathy; (ii) dysexecutive features, specifically poor planning, forethought, reasoning or organization; and (iii) disorders of language and communication. None of the patients had a pathology-proven diagnosis in the present study.

Patients were recruited from the Department of Neurology, Chiba University Hospital, Chiba, Japan. Seven patients with corticobasal syndrome, 12 with progressive supranuclear palsy, eight with frontotemporal dementia and 16 age-matched healthy controls were enrolled, after exclusion of two patients with corticobasal syndrome (poor PET imaging quality) and one control subject (poor arterial input). None of the subjects had a history of taking anticholinergic, acetylcholinesterase inhibitor medication, or substance abuse. None of the subjects had cerebrovascular disease, past history of head trauma or encephalitis. Medications were discontinued more than 12 h before performing the PET scans. Mini-Mental State Examination (MMSE) was administered to all subjects. Parkinsonism was rated with Unified Parkinson's Disease Rating Scale (UPDRS) motor score in patients with progressive supranuclear palsy.

This study was approved by the Institutional Review Board of the National Institute of Radiological Sciences. Written consent was obtained from all participants and their family members.

Positron emission tomography

After 10 min of transmission scan, emission data were acquired with a dynamic sequence of 16 PET scans over a 40 min period, using an EXACT47 scanner (Siemens/CTI, Knoxville, TN) with eyes covered and head immobilized. [^{11}C]MP4A (18.9 ± 2.8 mCi in 5 ml) was injected intravenously for 60 s using an infusion pump. Twenty-seven timed arterial blood samples were drawn from the radial artery. All emission scans were reconstructed by Hanning filter with a cut-off frequency of one-half of the maximum. After reconstruction, spatial resolution was 9 × 9 × 6 mm full-width at half maximum. Metabolite-corrected arterial plasma input function was obtained by fitting an exponential function to the plasma [^{11}C]MP4A radioactivity as described previously (Iyo *et al.*, 1997; Shinotoh *et al.*, 1999).

Data analysis

Image preprocessing was performed using Statistical Parametric Mapping software (SPM2, Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB 7.1 (Mathworks, Sherborn, MA). Each frame was realigned and individual mean images were created. In the corticobasal syndrome group, images were right-left flipped when the predominant side of clinical symptoms was on the left, so that the predominantly affected hemisphere was on the left side. [^{11}C]MP4A template image was created by using PET images and T_1-weighted MRI in 11 healthy controls according to standard methods (Meyer *et al.*, 1999). Each realigned image was individually normalized to the [^{11}C]MP4A template image by using the mean image as a target. These images were finally smoothed with a 12 mm full width at half maximum Gaussian kernel filter.

Custom software operating with the IDL image analysis software (Research Systems Inc., Boulder, CO) was employed to calculate the kinetic parameters in each voxel, and subsequently to generate individual parametric images. Kinetic parameters, K_1 (ml/min/g) (tracer transport into tissue), k_2 (min^{-1}) (tissue clearance of authentic tracer back to blood) and k_3 (min^{-1}) (hydrolysis rate from [^{11}C]MP4A to *N*-[^{11}C] methylpiperidinol, i.e. acetylcholinesterase activity) were estimated in a two-tissue three-parameter model by fitting brain theoretical curves to observed brain PET data, using an unconstrained non-linear least-squares method and weighted time-activity curve as described elsewhere (Iyo *et al.*, 1997; Shinotoh *et al.*, 1999).

Volumes of interest were placed on normalized parametric images by using the WFU Pickatlas (Maldjian *et al.*, 2003). The k_3 values were analysed in cerebral cortex and thalamus but not in cerebellum and striatum. Acetylcholinesterase activity in cerebellum and striatum is much higher than in cerebral cortex and thalamus. As a consequence, estimates of k_3 values in cerebellum and striatum are highly variable, and therefore not reliable, because of the flow-limitation effect of this technique (Namba *et al.*, 1999). Mean cortical k_3 value was calculated by averaging those in all Brodmann's areas, in each subject. The brain was left-right flipped in patients with corticobasal syndrome, when the more affected side was the left side of the body. Therefore, the left brain was the more affected side in all patients with corticobasal syndrome in the following analysis. Asymmetry index of K_1 and k_3 values for each participant was calculated as follows:

$$\text{Asymmetry index} = \frac{\text{Left cortex} - \text{Right cortex}}{\text{Left cortex} + \text{Right cortex}} \times 200.$$

Statistical analysis

All Statistical Parametric Mapping analyses were conducted without performing grand mean scaling (i.e. absolute value). For voxel-based whole brain analysis, we created an explicit cerebrum mask by using the Talairach Daemon template implemented into the WFU Pickatlas to increase power by excluding extracerebral white matter (Lancaster *et al.*, 2000; Maldjian *et al.*, 2003). Cerebellum and striatum were also excluded from the present analyses since, due to its ceiling effect, this technique does not allow for estimation in these brain areas. Comparisons between each disease group and healthy controls were performed with the unpaired *t*-test option. Clusters were accepted as significant if *P* values corrected for multiple comparisons were 0.05 or less on the cluster-level with extent threshold above 400 voxels in k_3 parametric analyses. The exploratory threshold for voxel-based analyses in K_1 parametric images was chosen ($P<0.001$ uncorrected, >400 voxels), since reduced perfusion in corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia was previously reported (Miller *et al.*, 1991; Markus *et al.*, 1995; Okuda *et al.*, 2000; Zhang *et al.*, 2001; Hossain *et al.*, 2003; Le Ber *et al.*, 2006; McNeill *et al.*, 2007). All reported coordinates were in Montreal Neurological Institute standard space.

Group comparisons in demographic variables and volume of interest analyses were performed by one-way ANOVA followed by Bonferroni correction, using Statistical Package for the Social Sciences software (SPSS version 11, SPSS Inc., Chicago, IL). Correlation analyses between cortical k_3 values and total MMSE scores were performed in each group by Spearman's rank correlation test. Correlation analysis between thalamic k_3 values, UPDRS motor score and axial subscore of UPDRS motor score (items 27–31) was also performed in patients with progressive supranuclear palsy.

Results

Patients

Demographic values are shown in Table 1. Age [$F(3,39)=4.37$, $P=0.10$, one-way ANOVA] and gender ($P=0.79$, chi-square) did not differ among disease and control groups (Table 1). MMSE scores differed among groups [$F(3.39)=14.4$, $P<0.001$] and *post hoc* analysis showed reduction of MMSE scores in the corticobasal syndrome ($P<0.005$) and frontotemporal dementia groups compared with the control group ($P<0.001$) (Table 1). Four patients with corticobasal syndrome were affected more on the right side and three patients with corticobasal syndrome more on the left side. The clinical features in corticobasal syndrome were: limb akinesia/rigidity 6/7; axial rigidity 4/7; myoclonic tremor 3/7; alien limb 4/6; supranuclear gaze palsy 3/7; and cortical sensory loss 3/4. All subjects with progressive supranuclear palsy presented with both supranuclear palsy and axial rigidity. Three patients with corticobasal syndrome and 10 patients with progressive supranuclear palsy were unresponsive to levodopa, and the remaining patients were not exposed to dopaminergic medications before the present study. The frontotemporal dementia group included six patients with behavioural variants of frontotemporal dementia, one patient with frontotemporal dementia with motor neuron disease and one patient with semantic

Table 1 Subject demographics

	n	M:F	Age (years)	Disease duration (years)	MMSE
Corticobasal syndrome	7	2:5	67.6 (5.9)	3.3 (1.8)	20.0 (8.4)
Progressive supranuclear palsy	12	5:7	68.5 (4.1)	4.3 (3.0)	25.1 (3.1)
Frontotemporal dementia	8	4:4	59.8 (6.9)	2.6 (1.6)	14.3 (8.9)
Healthy controls	16	8:8	61.2 (8.5)	–	28.9 (1.4)

Data are mean (SD).

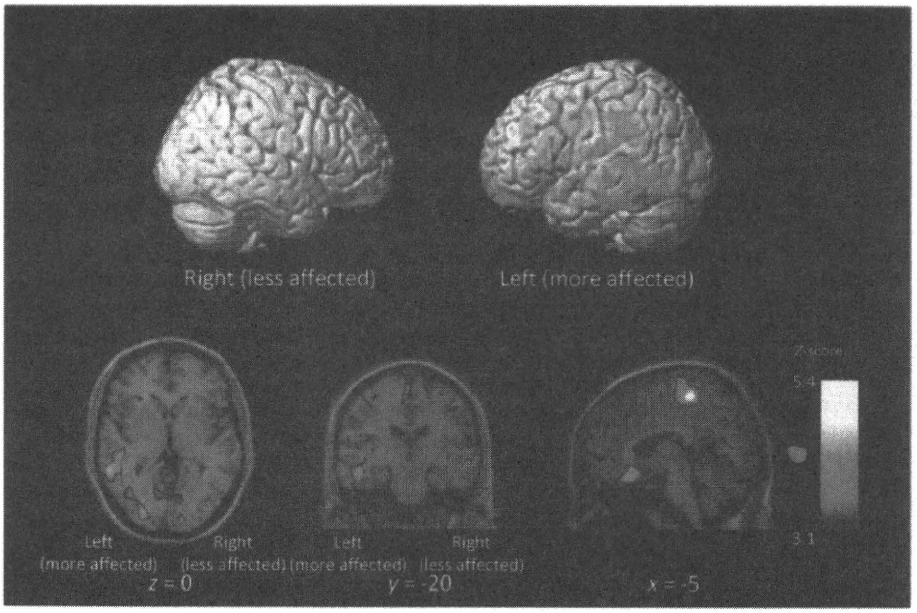

Figure 1 Statistical parametric *Z*-score map showing reduction of k_3 values in the brains of patients with corticobasal syndrome compared with healthy control brains at the threshold of $P<0.05$ with cluster correction. The brain is left-right flipped when the more affected side is the left side of the body, making the left brain the more affected side. Upper figure is rendered onto structural brain template. Lower figure is superimposed on brain MRI with different section (red–yellow).

dementia. There was no parkinsonism in the patients with frontotemporal dementia.

Acetylcholinesterase activity in disease groups compared with healthy controls

First, we compared the parametric k_3 brain images of disease and control groups ($P<0.05$, cluster-corrected). In corticobasal syndrome, there was a reduction of k_3 value in prefrontal, orbitofrontal, parietal and occipital cortices and the paracentral region compared with the control group (Fig. 1, Table 2). The most prominent reduction in k_3 value for corticobasal syndrome was observed in the precentral region (Brodmann's area 4) of the predominantly affected hemisphere. In progressive supranuclear palsy, reduction of k_3 values was detected in paracentral region and thalamus compared with the control group (Fig. 2, Table 2). There was no cluster with reduced k_3 values in the frontotemporal dementia group compared with healthy control brains. There was no cluster with increased k_3 values in any of the disease groups compared with healthy controls.

Volume of interest analysis demonstrated a significant difference in mean cortical k_3 values among groups [$F(3,39)=7.41$, $P<0.001$, one-way ANOVA] (Fig. 3A). *Post hoc* Bonferroni analysis revealed that, when compared with the healthy control group, mean cortical k_3 values were reduced in both corticobasal syndrome ($P<0.001$, –17.5% reduction) and progressive supranuclear palsy groups ($P<0.05$, –9.4% reduction), whereas mean cortical k_3 values in frontotemporal dementia did not differ from the healthy control group ($P=1.0$, –4.4% reduction). Mean cortical k_3 values in corticobasal syndrome were reduced compared with the frontotemporal dementia group ($P<0.04$). Thalamic k_3 values were also different among groups [$F(3,39)=3.99$, $P<0.02$, one-way ANOVA] (Fig. 3B). *Post hoc* Bonferroni analysis demonstrated that thalamic k_3 values were reduced in the progressive supranuclear palsy group compared with the healthy controls ($P<0.03$, –24.0% reduction). Thalamic k_3 values in the corticobasal syndrome group

Table 2 Comparison of brain acetylcholinesterase activity (k_3 value) in patients compared with healthy controls

Regions	Brodmann's area	Coordinates			P-value	Extent	Z_{max}
		x	y	z			
Controls > corticobasal syndrome							
Left paracentral region*	4, 6	−4	−34	62	<0.001	1565	5.39
Bilateral superior frontal and orbitofrontal lobe*	10, 11, 25	10	56	−24	<0.001	4947	5.31
Left paracentral region, precuneus, occipital lobe*	1–4, 6, 7, 17–21, 37, 40, 43	−14	−96	18	<0.001	12 148	5.26
Right parietal lobe	7, 39	26	−60	32	<0.05	452	4.8
Right parietal lobe	7	26	−48	52	<0.04	486	4.6
Right supplementary motor area	4, 6	36	−12	52	<0.002	1054	4.21
Right inferior occipital lobe	18	32	−86	−18	<0.02	659	4.15
Controls > progressive supranuclear palsy							
Right precentral region	4. 6	28	−2	60	<0.001	3020	4.72
Left paracentral region, insula, temporal lobe	1–4, 6, 13, 20, 21, 43, 44	−56	−2	22	<0.001	3465	4.62
Left postcentral region, parietal lobe	1–5	−14	−36	68	<0.001	4714	4.49
Left thalamus	–	−16	−28	0	<0.003	514	4.38

SPM, *P*-values corrected at the cluster level.
*$P < 0.05$ family-wise error corrected.

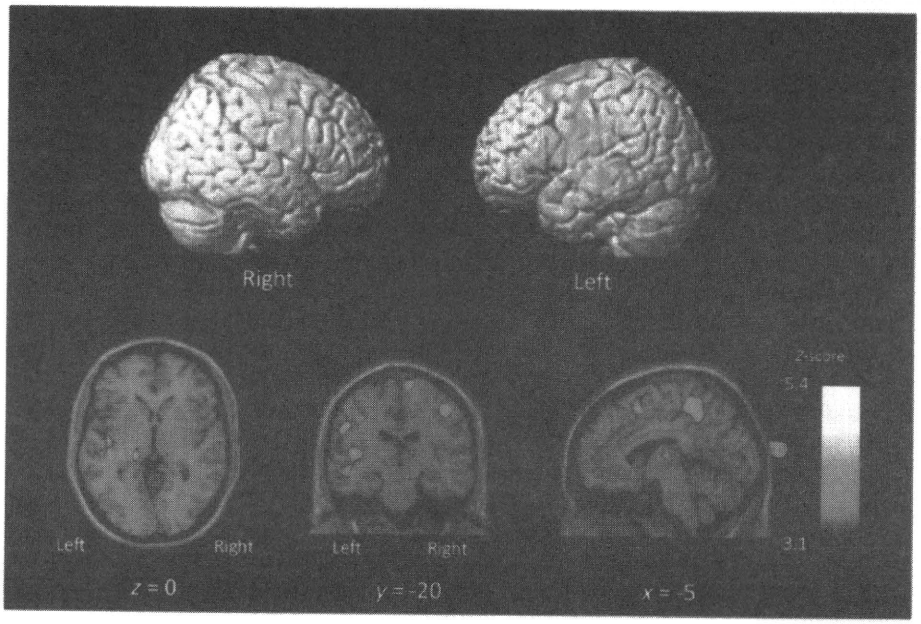

Figure 2 Statistical parametric *Z*-score map showing reduction of k_3 values in the brains of patients with progressive supranuclear palsy compared with healthy control brains at the threshold of $P < 0.05$ with cluster correction. Upper figure is rendered onto structural brain template. Lower figure is superimposed on brain MRI with different section (red–yellow).

($P = 1.0$, −6.4% reduction) and frontotemporal dementia group ($P = 1.0$, 3.3% increase) did not differ from the control group. Comparison of disease groups in terms of thalamic k_3 values showed reduction in the progressive supranuclear palsy group compared with the frontotemporal dementia group ($P < 0.04$).

Correlation analysis between mean cortical k_3 values and MMSE total scores in each group revealed that there was a positive correlation only in the corticobasal syndrome group (Spearman's rho = 0.8, $P < 0.04$), but not in the progressive supranuclear palsy or frontotemporal dementia group ($P \geq 0.60$). Thalamic k_3 values were not correlated with UPDRS motor scores or their axial subscores in patients with progressive supranuclear palsy ($P > 0.05$).

Changes in perfusion of corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia

There was reduction in K_1 values in the corticobasal syndrome, progressive supranuclear palsy and frontotemporal dementia

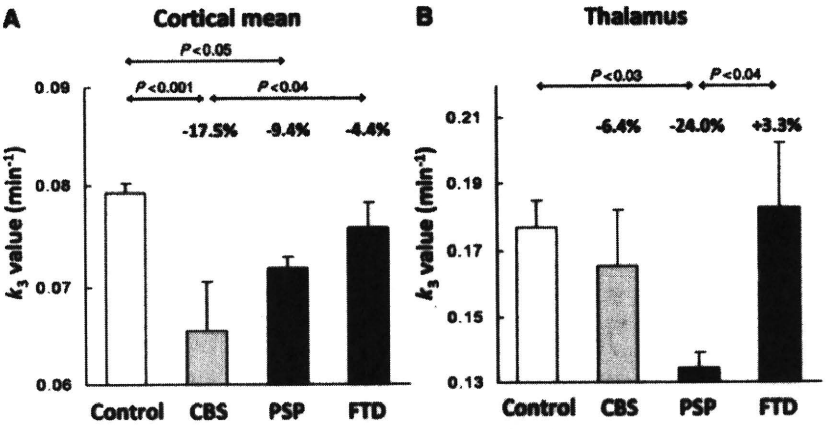

Figure 3 Graphs of k_3 value, an index for brain acetylcholinesterase activity, in healthy controls, patients with corticobasal syndrome (CBS), progressive supranuclear palsy (PSP) and frontotemporal dementia (FTD) (mean ± SEM). (**A**) Cortical mean k_3 values are reduced in corticobasal syndrome ($P<0.001$) and progressive supranuclear palsy ($P<0.05$) whereas those in frontotemporal dementia are not different from controls. (**B**) Thalamic k_3 value is reduced in patients with progressive supranuclear palsy compared with healthy controls ($P<0.03$). Thalamic k_3 value is preserved in corticobasal syndrome and frontotemporal dementia ($P=1.00$).

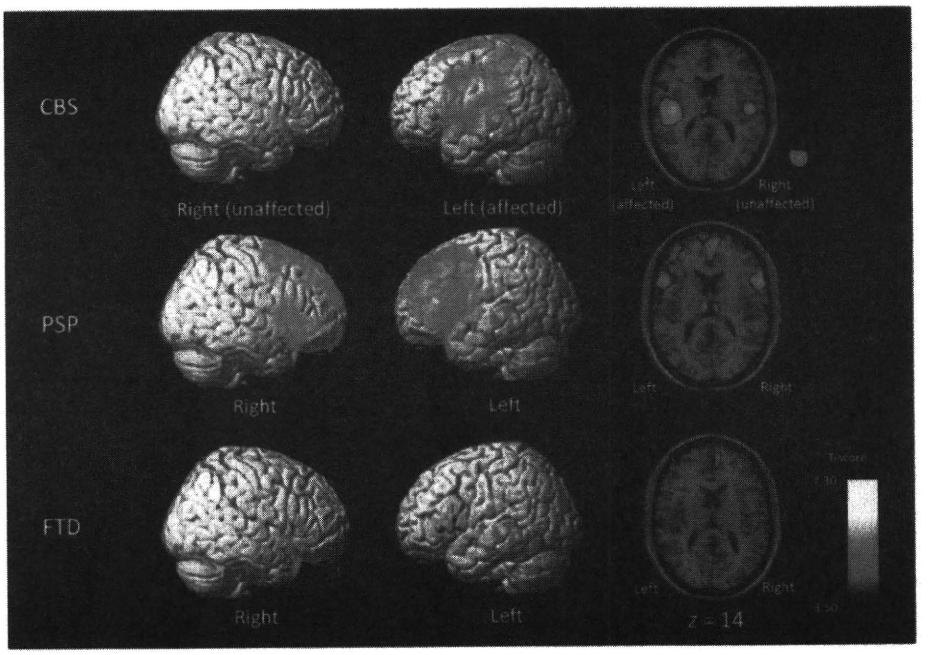

Figure 4 Statistical parametric Z-score map showing reduction of K_1 values in the brains of patients with corticobasal syndrome (CBS), progressive supranuclear palsy (PSP) and frontotemporal dementia (FTD) compared with healthy control brains at the threshold of $P<0.001$, uncorrected, extent threshold > 400 voxels. Left figure is rendered onto structural brain templa... Right figure is superimposed on brain MRI with different section (red–yellow).

groups compared with the control group (Fig. 4, $P<0.001$, voxel-uncorrected, extent threshold > 400). In corticobasal syndrome, there was asymmetric reduction of K_1 values, with more reduction on the predominantly affected side of the brain than the less affected side, in paracentral and parietal region. The progressive supranuclear palsy group showed reduction of K_1 values in frontotemporal cortices and thalamus. There was reduction of K_1 values in the mesial frontal and right temporal region in the frontotemporal dementia group. Detailed results of coordinates and Z-values are presented in the online Supplementary material.

Laterality of K_1 and k_3 changes

The K_1 and k_3 values in each volume of interest are shown in Table 3. There was reduction of k_3 values in bilateral frontal, temporal, parietal and occipital cortices in the corticobasal syndrome group, while there was reduction of K_1 values only in the more affected brain hemispheres in the corticobasal syndrome group. Asymmetry index for K_1 and k_3 values in cerebral cortex and thalamus was calculated and compared between groups (Table 3). Asymmetry index for cortical k_3 tended to be high in the corticobasal syndrome group, but there was no main effect for cortical k_3 values [$F(3,39) = 1.31$, $P = 0.29$] or thalamic k_3 value [$F(3,39) = 1.48$, $P = 0.23$]. No significant left/right differences were found in cortical and thalamic acetylcholinesterase activity in any of the disease groups, including the corticobasal syndrome group. There was a main effect for cortical K_1 values [$F(3,39) = 4.79$, $P < 0.007$]. *Post hoc* Bonferroni test showed a difference in asymmetric index of cortical K_1 values between corticobasal syndrome and frontotemporal dementia ($P < 0.004$) and a trend towards difference between corticobasal syndrome and control group ($P = 0.10$). There was no main effect for thalamic K_1 value [$F(3,39) = 2.75$, $P = 0.06$]. A trend of difference in thalamic K_1 value was found between the controls and corticobasal syndrome group ($P = 0.06$), decreased in the affected side of corticobasal syndrome group.

Discussion

To our knowledge, this is the first *in vivo* study of brain cholinergic function in patients with corticobasal syndrome and frontotemporal dementia. Cerebral cortical acetylcholinesterase activity was moderately reduced in corticobasal syndrome and mildly reduced in progressive supranuclear palsy, while thalamic acetylcholinesterase activity was remarkably reduced only in progressive supranuclear palsy. There was no acetylcholinesterase activity deficit in cerebral cortex and thalamus in the frontotemporal dementia group. Cortical acetylcholinesterase activity was correlated with general cognitive function measured by MMSE in the corticobasal syndrome group. There was a trend toward lateralizing asymmetry in cortical acetylcholinesterase reduction that was more severe in the more affected brain hemisphere and regionally more severe in the parieto-occipital and orbitofrontal region in the corticobasal syndrome group. Deficits in acetylcholinesterase activity in cerebral cortex were symmetrical in the progressive supranuclear palsy and frontotemporal dementia groups.

Pathological studies of post-mortem brains with corticobasal syndrome showed that the nucleus basalis of Meynert is relatively preserved and contains a few neurofibrillary tangles (Dickson, 1999; Dickson *et al.*, 2002). While abnormal tau processing is limited to grey matter in Alzheimer's disease, white matter is

Table 3 **Acetylcholinesterase activity (k_3) and perfusion (K_1) value in volume of interest analysis[a] and asymmetry index[b]**

		Control	Corticobasal syndrome	Progressive supranuclear palsy	Frontotemporal dementia
k_3	Frontal, right	0.082 (0.001)	0.069 (0.005), −15.8%[c]	0.074 (0.002), −9.7%[c]	0.078 (0.002), −4.7%
	Frontal, left	0.081 (0.001)	0.067 (0.006), −17.6%[c]	0.074 (0.002), −8.6%	0.077 (0.003), −5.5%
	Temporal, right	0.076 (0.002)	0.065 (0.005), −15.2%[c, d]	0.070 (0.001), −8.0%	0.076 (0.003), +0.3%
	Temporal, left	0.078 (0.001)	0.062 (0.006), −21.1%[c, d]	0.071 (0.002), −9.0%	0.079 (0.005), +0.9%
	Parietal, right	0.072 (0.001)	0.061 (0.005), −15.8%[c]	0.066 (0.001), −8.2%	0.070 (0.002), −3.5%
	Parietal, left	0.073 (0.001)	0.059 (0.006), −18.8%[c, d]	0.067 (0.001), −8.7%	0.071 (0.002), −2.8%
	Occipital, right	0.064 (0.001)	0.053 (0.003), −16.8%[c, d]	0.058 (0.002), −9.0%	0.061 (0.001), −4.8%
	Occipital, left	0.067 (0.001)	0.054 (0.004), −18.9%[c, d]	0.061 (0.002), −9.0%	0.066 (0.002), −2.4%
	Thalamus, right	0.170 (0.008)	0.165 (0.015), −3.1%	0.134 (0.006), −21.3%[d]	0.193 (0.024), +13.4%
	Thalamus, left	0.184 (0.011)	0.167 (0.019), −9.1%	0.135 (0.005), −26.4%[c]	0.173 (0.017), −6.0%
	Cortex AI	1.14 (0.57)	−3.24 (1.84)	1.33 (1.94)	0.94 (2.74)
	Thalamus AI	6.81 (4.66)	0.28 (5.17)	1.39 (3.62)	−9.12 (8.29)
K_1	Frontal, right	0.396 (0.017)	0.324 (0.017), −18.0%	0.316 (0.018), −20.0%[c]	0.334 (0.027), −15.7%
	Frontal, left	0.394 (0.017)	0.303 (0.017), −22.9%[c]	0.313 (0.017), −20.4%[c]	0.351 (0.026), −10.8%
	Temporal, right	0.401 (0.017)	0.338 (0.020), −15.7%	0.339 (0.020), −15.3%	0.349 (0.033), −12.9%
	Temporal, left	0.409 (0.017)	0.310 (0.023), −24.3%[c]	0.341 (0.019), −16.7%	0.381 (0.030), −6.9%
	Parietal, right	0.413 (0.016)	0.332 (0.021), −19.6%	0.360 (0.019), −12.6%	0.381 (0.030), −7.7%
	Parietal, left	0.419 (0.017)	0.307 (0.021), −26.8%[c, d]	0.361 (0.017), −13.9%	0.411 (0.028), −1.9%
	Occipital, right	0.383 (0.015)	0.337 (0.017), −12.1%	0.328 (0.017), −14.5%	0.380 (0.025), −0.8%
	Occipital, left	0.398 (0.015)	0.332 (0.021), −16.6%	0.338 (0.017), −15.2%	0.397 (0.026), −0.4%
	Thalamus, right	0.483 (0.021)	0.398 (0.020), −17.6%	0.382 (0.002), −21.1%[c]	0.436 (0.035), −9.9%
	Thalamus, left	0.492 (0.020)	0.375 (0.021), −23.9%[c]	0.387 (0.021), −21.3%[c]	0.445 (0.043), −9.6%
	Cortex AI	1.01 (0.59)	−6.78 (2.41)[d]	0.45 (0.70)	6.67 (5.09)
	Thalamus AI	1.91 (1.46)	−6.24 (1.48)	1.33 (1.19)	0.77 (3.98)

K_1: ml/min/g, k_3: min^{-1}.
Asymmetry index (AI) = (left cortex − right cortex)/(left cortex + right cortex) × 200.
The brain is left-right flipped in corticobasal syndrome group, when the more affected side is the left side of the body.
a Mean (SEM), percent reduction relative to mean of healthy control group.
b Mean (SEM).
c Reduced compared to healthy controls ($P < 0.05$), one-way ANOVA, *post hoc* Bonferroni.
d: Reduced compared to frontotemporal dementia ($P < 0.05$), one-way ANOVA, *post hoc* Bonferroni.

also involved in addition to grey matter in corticobasal syndrome (Dickson, 1999). Although asymmetric cortical atrophy is characteristic of corticobasal syndrome (Soliveri *et al.*, 1999; Zadikoff and Lang, 2005), a neurochemical study has shown that insoluble tau is symmetrically and equally distributed between grey and white matter in corticobasal syndrome (Forman *et al.*, 2002). White matter change has been detected by structural MRI studies showing subcortical T_2 high intensity in primary and supplementary motor cortices (Soliveri *et al.*, 1999; Winkelmann *et al.*, 1999) and a study of diffusion tensor tractography demonstrated reduction of corticospinal tract in corticobasal syndrome (Boelmans *et al.*, 2009). Taken together, the reduction of cortical acetylcholinesterase activity in corticobasal syndrome could be explained by impairment of cholinergic fibres from the nucleus basalis of Meynert or dysfunction of cholinergic neurons without significant loss of cholinergic neurons in the nucleus basalis of Meynert.

Perfusion, an index of cortical neuronal activity, was asymmetrically reduced in cerebral cortex of the corticobasal syndrome group in the present study, which is in accord with previous PET studies showing asymmetric hypoperfusion or hypometabolism in the frontal, parietal, temporal cortices, basal ganglia and thalamus (Markus *et al.*, 1995; Okuda *et al.*, 1999, 2000; Brooks, 2000; Zhang *et al.*, 2001; Hossain *et al.*, 2003; Eckert *et al.*, 2005). We found a correlation between MMSE scores and cortical acetylcholinesterase activity in the corticobasal syndrome group, suggesting that cognitive decline might be caused by cholinergic dysfunction in corticobasal syndrome. Cholinergic stimulant therapy may improve cognitive function of corticobasal syndrome patients.

Loss of cortical acetylcholinesterase activity was mild and loss of thalamic acetylcholinesterase activity was pronounced in progressive supranuclear palsy. These results are concordant with that of our previous [^{11}C]MP4A PET study using manual regions of interest (Shinotoh *et al.*, 1999). Post-mortem progressive supranuclear palsy brain studies showed that neuronal cell loss in the nucleus basalis of Meynert was variable, ranging from –12.6 to –73.8% less than those in Parkinson's disease and Alzheimer's disease (Tagliavini *et al.*, 1984; Rogers *et al.*, 1985; Ruberg *et al.*, 1985). Neurochemical and histochemical studies of post-mortem brains showed that choline acetyltransferase activity is only mildly reduced in frontal cortex of progressive supranuclear palsy (Ruberg *et al.*, 1985; Suzuki *et al.*, 2002). The mild reduction of acetylcholinesterase activity in the progressive supranuclear palsy group in the present study is in agreement with those results from the post-mortem brain studies. In another PET study with ^{11}C-*N*-methyl-4-piperidyl benzilate, we demonstrated that there was no significant change in total muscarinic acetylcholine receptors in cerebral cortex and thalamus in patients with progressive supranuclear palsy, suggesting that cholinoceptive neurons are not impaired in cerebral cortex or thalamus in progressive supranuclear palsy (Asahina *et al.*, 1998). Neuropathological studies have demonstrated severe loss of pedunculopontine nucleus neurons in progressive supranuclear palsy (Hirsch *et al.*, 1987; Zweig *et al.*, 1987; Jellinger, 1988; Warren *et al.*, 2005). Taken together, the pronounced reduction of thalamic acetylcholinesterase activity in patients with progressive supranuclear palsy is thought to reflect a loss of ascending cholinergic fibres from pedunculopontine and laterodorsal tegmental nuclei rather than impairment of cholinoceptive neurons in thalamus, although we did not find any significant correlation between thalamic k_3 values and UPDRS motor scores or axial scores of UPDRS in the present study. The impairment of pedunculopontine nucleus can produce disturbances in gait, posture, eye movement and attention (Masdeu *et al.*, 1994), which are main clinical features in progressive supranuclear palsy.

Since cortical involvement in progressive supranuclear palsy is sparse, behavioural disturbances have previously been attributed to disconnection of the frontal cortex by prominent subcortical pathology (Agid *et al.*, 1987; Litvan *et al.*, 1996). Our results suggest that dysfunction of the thalamocortical pathway could also contribute to the neuropsychological impairment in progressive supranuclear palsy.

In the present study, there was a reduction in frontal cortex and thalamus perfusion in the progressive supranuclear palsy group, a result in accord with previous studies showing reduction of perfusion and glucose metabolism in prefrontal cortex, peri-insula, caudate nucleus, thalamus and mesencephalon (D'Antona *et al.*, 1985; Salmon *et al.*, 1997; Garraux *et al.*, 1999; Hosaka *et al.*, 2002; Van Laere *et al.*, 2006; Eckert *et al.*, 2008).

In spite of the mounting evidence of cholinergic impairment in the brain of patients with progressive supranuclear palsy, a number of drug trial studies have failed to show beneficial effects of cholinergic stimulant therapy (Foster *et al.*, 1989; Litvan *et al.*, 1989, 2001; Fabbrini *et al.*, 2001). However, in the donepezil study, patients with progressive supranuclear palsy who did not receive dopaminergic therapy showed better memory scores on the category cued recall (Litvan *et al.*, 2001). Additionally, a physostigmine trial in a small group of patients with progressive supranuclear palsy showed increased brain metabolism and some improvement in memory and visuospatial attention (Kertzman *et al.*, 1990; Blin *et al.*, 1995). On the contrary, brain cholinergic blockade by scopolamine significantly impairs cognitive and gait function in progressive supranuclear palsy by increased sensitivity (Litvan *et al.*, 1994). The present study confirmed the brain cholinergic deficits in progressive supranuclear palsy. Other forms of acetylcholine modulating agent might be helpful for improving clinical symptoms in patients with progressive supranuclear palsy.

Accumulating neuropathological evidence has indicated that both cortical acetylcholinesterase and choline acetyltransferase are unaltered, even in the advanced stage of frontotemporal dementia (White *et al.*, 1977; Yates *et al.*, 1980; Wood *et al.*, 1983; Meier-Ruge *et al.*, 1984; Hansen *et al.*, 1988; Sparks and Markesbery, 1991; Procter *et al.*, 1999). A study of cerebrospinal fluid in frontotemporal dementia demonstrated a normal acetylcholinesterase level (Wallin *et al.*, 2003). Our result in the frontotemporal dementia group agreed with this. A therapeutic trial of acetylcholinesterase inhibitor in frontotemporal dementia failed to show any efficacy (Mendez *et al.*, 2007). Dementia and behavioural abnormalities in frontotemporal dementia are probably related to impairment of systems other than the cholinergic system. The present frontotemporal dementia group showed reduction of perfusion in the mesial frontal and left dorsolateral frontal regions. Previous perfusion single photon emission computed tomography studies have repeatedly shown frontal and

anterior temporal reduction and relative preservation of parietal and occipital cortices (Miller *et al.*, 1991; Le Ber *et al.*, 2006; McNeill *et al.*, 2007). Although the reduction of frontotemporal perfusion was modest in patients with frontotemporal dementia, our result is in agreement with those previous observations, supporting the diagnoses of patients with frontotemporal dementia in the present study. Patients with frontotemporal dementia had to be either calm or inert enough to stay still in the PET scanner bed for nearly an hour. Patients with frontotemporal dementia in this study were in the early stage of the disease (mean disease duration 2.6 years). This might have resulted in modest frontotemporal hypoperfusion compared with previous perfusion studies.

In contrast to the frontotemporal dementia group in this study, we previously observed profound reduction of acetylcholinesterase activity in cerebral cortex and thalamus in two patients with frontotemporal dementia and parkinsonism linked to chromosome-17 (FTDP-17) who had a mutation (*N279K*) in the microtubule-associated protein tau (*MAPT*) gene (Hirano *et al.*, 2006). The pathologies and the clinical features of FTDP-17 with *MAPT* gene mutation mimic corticobasal syndrome (Bird *et al.*, 1999; Nasreddine *et al.*, 1999; Dickson *et al.*, 2002). Interestingly, FTDP-17 with *MAPT* gene mutation is also characterized by four-repeat tau deposition along with corticobasal syndrome and progressive supranuclear palsy, while Pick's disease is characterized predominantly by three-repeat tau deposition. Frontotemporal dementia with motor neuron disease and semantic dementia do not usually show tau pathology. Corticobasal syndrome and progressive supranuclear palsy are closer to FTDP-17 than frontotemporal dementia, including Pick's disease (Boeve *et al.*, 2003), which may explain the difference in the cholinergic pathology in these disorders.

We have studied brain acetylcholinesterase activity in other neurodegenerative diseases by PET and found that mean reduction of cortical acetylcholinesterase activity, compared with normal controls, was 13% in mild to moderate late-onset Alzheimer's disease, 23% in mild to moderate early-onset Alzheimer's disease, 12% in Parkinson's disease without dementia, 27% in Parkinson's disease with dementia and dementia with Lewy bodies, 21 and 36% in two patients with N279K FTDP-17 and 6% in a cerebellar variant of multiple system atrophy (Shinotoh *et al.*, 2000; Hirano *et al.*, 2006, 2008; Shimada *et al.*, 2009). Compared with the reduction of cortical acetylcholinesterase activities in these disorders, the reduction of cortical acetylcholinesterase was moderate in corticobasal syndrome and mild in progressive supranuclear palsy.

The limitation of the present study is the lack of pathological data from the patients. There is growing evidence that patients with clinically diagnosed corticobasal syndrome can have alternative pathologies including Alzheimer's disease, progressive supranuclear palsy and frontotemporal dementia (Boeve *et al.*, 1999; Kertesz *et al.*, 2000; Josephs *et al.*, 2006; Sha *et al.*, 2006). Nevertheless, as we included only patients with corticobasal syndrome who were diagnosed on the basis of movement disorder, and none of them had initial episodic memory impairment, the pathological diagnosis of corticobasal degeneration rather than Alzheimer's disease was strongly suggested. Pathological diagnosis could overlap, but it is unlikely to have a cholinergic deficit in frontal syndromes when Parkinsonian features are absent.

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Supplementary material

Supplementary material is available at *Brain* online.

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Estimation of Plasma IC_{50} of Donepezil for Cerebral Acetylcholinesterase Inhibition in Patients With Alzheimer Disease Using Positron Emission Tomography

Tsuneyoshi Ota, PhD, MD,† Hitoshi Shinotoh, PhD, MD,*‡ Kiyoshi Fukushi, PhD,* Tatsuya Kikuchi, PhD,* Koichi Sato, PhD, MD,*§ Noriko Tanaka, PhD, MD,*|| Hitoshi Shimada, PhD, MD,*¶ Shigeki Hirano, PhD, MD,*# Michie Miyoshi, MD,* Heii Arai, PhD, MD,† Tetsuya Suhara, PhD, MD,* and Toshiaki Irie, PhD**

Objectives: Estimate the value of in vivo plasma IC_{50} of donepezil, the concentration of donepezil in plasma that inhibits brain acetylcholinesterase (AChE) activity by 50% at the steady-state conditions of donepezil between the plasma and the brain.

Methods: *N*-[^{11}C] methylpiperidin-4-*yl* acetate ([^{11}C]MP4A) positron emission tomography was performed in 16 patients with probable Alzheimer disease (AD) before and during the treatment of donepezil (5 mg/day) with a mean interval of 5.3 months. The plasma IC_{50} value of donepezil was estimated from plasma donepezil concentrations and cerebral cortical mean AChE inhibition rates measured by positron emission tomography, using one-parameter model.

Results: Donepezil reduced AChE activity uniformly in the cerebral cortex compared with the baseline in each AD patient, and the mean reduction rate in the cerebral cortex was 34.6%. The donepezil concentrations in the plasma ranged from 18.5 to 43.9 ng/mL with a mean of 28.9 ± 7.3 ng/mL. The plasma IC_{50} value was estimated to be 53.6 ± 4.0 ng/mL.

Conclusions: Once the plasma IC_{50} of donepezil is determined, the brain AChE inhibition rate could be estimated from the plasma concentration of donepezil in each subject based on the plasma IC_{50}. Now that the mean donepezil concentrations in the plasma, when the patients took 5 mg/day, remained 28.9 ng/mL, approximately half of the plasma IC_{50}, higher dose of donepezil might provide further benefits for patients with AD. This technique can be also applied to measure the in vivo plasma IC_{50} of other cholinesterase inhibitors such as rivastigmine and galantamine.

Key Words: acetylcholinesterase, Alzheimer disease, donepezil, in vivo plasma IC_{50}, positron emission tomography

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Donepezil hydrochloride, a specific acetylcholinesterase (AChE) inhibitor, is widely used for the treatment of Alzheimer disease (AD). Donepezil inhibits AChE and increases concentrations of acetylcholine in the synaptic cleft. This is believed to contribute to the cognitive improvement in patients with AD during treatment with donepezil.[1] Donepezil has also come to be more expected as a disease-modifying drug.[2–4] Until recently, patients with AD in Japan were treated with an identical dose (5 mg/day). Now that 10 mg of donepezil is also permitted in Japan, the decision of the appropriate clinical dosage of donepezil is necessary.

N-[^{11}C]methylpiperidin-4-*yl* acetate ([^{11}C]MP4A or AMP) and *N*-[^{11}C]methylpiperidin-4-*yl* propionate ([^{11}C]MP4P or PMP) are both acetylcholine analogues which were developed as positron emission tomography (PET) radiotracers for brain AChE mapping[5,6]; they have been applied to the quantification of cortical AChE activity in healthy subjects[7,8] and in patients with AD and other neurodegenerative disorders.[9–11] Both radiotracers have also been used to evaluate the inhibitory effects of donepezil on brain AChE activity in patients with AD[12–15] and in monkeys.[16,17]

Recently, the correlation between plasma galantamine concentrations and brain AChE inhibition rates was examined in patients with AD using [^{11}C]MP4P.[18] As for donepezil, however, there has been no in vivo human study on the quantitative correlation between the plasma donepezil concentrations and brain AChE inhibition rates. We investigated the correlation between plasma donepezil concentrations and AChE inhibition rates in the cerebral cortex using [^{11}C]MP4A PET in patients with probable AD before and during treatment. We also estimated in vivo plasma IC_{50} of donepezil, on the premise that the inhibitory effects of AChE inhibitors are dose dependent on AChE activity.[17,19] Subsequently, the AChE inhibition rates in the cerebral cortex can be estimated from the plasma concentration of donepezil in each patient with AD at various doses. This is the first report on the estimation of in vivo plasma IC_{50} of donepezil in patients with AD.

This study is an extension of the previous study in which we quantitatively measured cerebral cortical AChE activities in 3 patients with AD once before and once during donepezil treatment by [^{11}C]MP4A PET and showed that donepezil reduced AChE activity in the cerebral cortex by 39 ± 5%.[13]

PATIENTS AND METHODS

Subjects

Sixteen patients (5 men and 11 women ranging from 50 to 83 years old) with AD participated in this study. The patients' mean age was 66.0 ± 10.2 years, and the mean duration of illness was 2.7 ± 1.4 years at the time of the baseline PET study. The mean duration of treatment was 5.3 ± 2.0 months. Three patients with AD included in the previous report were also included in this study.[13] The patients had been taking neither anticholinergic

From the *Department of Molecular Probe, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba; †Department of Psychiatry, Juntendo University School of Medicine, Tokyo; ‡Asahi Hospital for Neurological Diseases; §Department of Psychiatry, Teikyo University Chiba Medical Center; ||Department of Neurosurgery, Tokyo Women's Medical University Medical Center East, Tokyo; ¶Department of Neurology, Chiba University School of Medicine, Chiba; and #Department of Neurology, JR Tokyo General Hospital, Tokyo, Japan.

Address correspondence and reprint requests to Tsuneyoshi Ota, PhD, MD, Department of Psychiatry, Juntendo University School of Medicine, Tokyo, Japan; E-mail: ota@juntendo.ac.jp

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medications nor AChE inhibitors other than donepezil before the PET study. All patients met the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria for probable AD.[20] The absence of focal cerebral abnormalities was confirmed by magnetic resonance imaging in all the patients.

This study was approved by the institutional review board of the National Institute of Radiological Sciences, Chiba, Japan. Written informed consent was obtained from each participant and/or his/her caregiver in the family before the study.

Calculation of Donepezil-Induced Inhibition of Cortical AChE Activity Using [^{11}C]MP4A PET

We performed [^{11}C]MP4A PET in 16 patients with probable AD in the baseline PET studies. The patients started to receive donepezil orally at a dose of 3 mg once a day for 2 weeks after the baseline PET study, followed by 5 mg daily for 5.3 ± 2.0 (mean ± SD) months until the second PET study. The patients took 5 mg of donepezil at 8 AM and underwent the follow-up PET study at 3 PM. All medications other than donepezil were withdrawn at least 12 hours before the PET study. A venous blood sample was obtained from each patient with AD for the measurement of the plasma concentration of donepezil around 3 PM just before tracer injection in the second PET study.

A sequence of 14 PET scan was acquired with EXACT 47 scanner (Siemens/CTI, Knoxville, Tenn) covering 40 minutes after IV injection of [^{11}C]MP4A (approximately 740 MBq) in each subject. Twenty-four arterial blood samples were collected at the predetermined intervals for 15 minutes after IV tracer injection for measurement of total radioactivity and metabolite analysis. A three-compartment model was used to yield estimation of K_1 (transport into tissue), k_2 (tissue clearance of unchanged tracer into blood), and k_3 (hydrolysis rate of [^{11}C]MP4A by AChE, ie, AChE activity) using metabolite-corrected arterial plasma input function. The voxel-by-voxel k_3 value was calculated with the arterial input function. The procedures in detail were previously described.[9,13,21] The voxels of interest were automatically determined in the bilateral frontal cortex (superior frontal gyrus and middle frontal gyrus), sensorimotor cortex (precentral and postcentral gyrus), temporal cortex (superior temporal gyrus), parietal cortex (inferior parietal gyrus), occipital cortex (middle occipital gyrus and cuneus), and in the posterior cingulate cortex using the analytic software, the voxel-based stereotactic extraction estimation, which determines anatomical information in respective brain coordinates[22] with the Talairach Daemon.[23] The regional cortical k_3 values and donepezil-induced inhibition rates were calculated. Percentage changes in k_3 value were defined by the following formula:

$$\%\ \text{change in}\ k_3 = \left(k_{3(b)} - k_{3(f)}\right) / k_{3(b)} \times 100$$

where $k_{3(f)}$ and $k_{3(b)}$ represent k_3 values at the time of follow-up and at baseline, respectively. Data in the striatum and cerebellum were not analyzed because k_3 estimates in these brain regions with high AChE activity are not reliable because of the characteristics of this technique.[9]

Estimation of Plasma IC_{50}

A venous blood sample was taken from each patient with AD for measurement of plasma concentration of donepezil just before tracer injection (around 3 PM) in the second PET study. The plasma components were separated immediately from the cellular components by centrifugation and stored at −20°C until the analysis. Plasma concentrations were measured using a specific and sensitive high-performance liquid chromatography method with ultraviolet detection. The lowest quantifiable concentration was 2.0 ng/mL.[24,25] We investigated the correlation between plasma donepezil concentrations and AChE inhibition rates in the cerebral cortex. Then, the value of in vivo plasma IC_{50} of donepezil, that is, the concentration of donepezil in plasma that inhibits brain AChE activity by 50% at the steady-state conditions of donepezil between the plasma and the brain, was calculated by measurement of plasma donepezil concentrations and the cerebral cortical AChE inhibition rates evaluated with [^{11}C]MP4A PET. The plasma IC_{50} was estimated using a simple one-parameter model as followed:

$$\text{AChE inhibition} = [\text{I}]/(\text{plasma IC}_{50} + [\text{I}])$$

where AChE inhibition represents mean k_3 reduction in the cortical voxels of interest defined above and [I] represents the donepezil concentration in the plasma.[17,26,27] The standard errors of plasma IC_{50} estimates were calculated with the variance-covariance matrix.

RESULTS

The steady-state plasma concentration of donepezil after oral doses of 5 mg/day was 28.9 ± 7.3 ng/mL after 5.3 ± 2.0 months of treatment in patients with AD. The inhibition levels of AChE activity in cerebral cortices caused by donepezil are shown in Table 1. The plasma concentration of donepezil ranged from 18.5 to 43.9 ng/mL with mean of 28.9 ± 7.3 ng/mL, and the inhibition rates of the cerebral cortical AChE activity ranged from 21.0% to 43.6% with mean of 34.6 ± 7.3% compared with those in the baseline study ($P < 0.0001$). The AChE activity was uniformly inhibited by donepezil among cortical regions as in the lateral temporal cortex (37.1%), followed by superior frontal (35.4%), sensorimotor (35.1%), posterior cingulate (33.1%), occipital (32.5%), and parietal (32.4%) cortices.

The plasma IC_{50} value estimated from plasma donepezil concentrations and the cerebral cortical mean AChE inhibition rates measured by PET was 53.6 ± 4.0 ng/mL (Fig. 1). The correlation between the plasma donepezil concentration and the AChE inhibition rate remained 0.50 ($P = 0.05$).

DISCUSSION

The PET evaluation provides unique information on the in vivo pharmacology of AChE inhibitors and novel drugs. Until the development of a PET technique for the measurement of

TABLE 1. The Regional Cortical k_3 Values (Mean ± SD) and Donepezil-Induced Inhibition Rate Measured by [^{11}C]MP4A PET

Region	k_3 (per minute)		Inhibition (%)
	Pretreatment	Posttreatment	
Frontal	0.0674 ± 0.0075	0.0433 ± 0.0058	35.4 ± 8.6*
Sensorimotor	0.0744 ± 0.0089	0.0481 ± 0.0069	35.1 ± 7.5*
Lateral temporal	0.0695 ± 0.0096	0.0435 ± 0.0074	37.1 ± 8.2*
Parietal	0.0566 ± 0.0077	0.0388 ± 0.0057	32.4 ± 8.6*
Occipital	0.0543 ± 0.0067	0.0365 ± 0.0053	32.5 ± 7.7*
Posterior cingulate	0.0584 ± 0.0062	0.0388 ± 0.0049	33.1 ± 9.9*
Cortical mean	0.0634 ± 0.0065	0.0414 ± 0.0055	34.6 ± 7.3*

Data are expressed as mean ± SD.

[^{11}C]MP4A indicates *N*-[^{11}C] methylpiperidin-4-yl acetate.

*$P < 0.0001$: significant reduction against pretreatment (2-tailed paired *t* test).

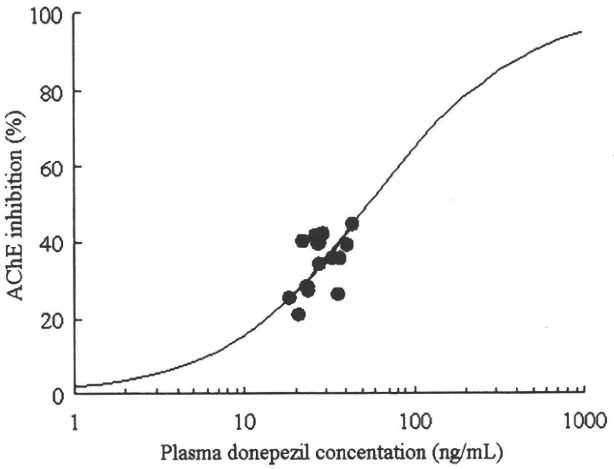

FIGURE 1. Relationship between the plasma donepezil concentration and the percent inhibition of brain AChE activity. According to the concentration-inhibition curve, the plasma IC_{50} value of donepezil is estimated at 53.6 ± 4.0 ng/mL (approximately 130 nM). Using the reported protein-bound fraction of donepezil in healthy human plasma, the plasma IC_{50} of donepezil in the free fraction is estimated to be 9.6 nM.

AChE activity, the relationship between AChE inhibitors and the extent of the central AChE inhibition process has been merely inferred from peripheral measures, such as by red blood cell (RBC) membrane AChE assays. The plasma concentration of donepezil is directly related to RBC AChE inhibition; it is reported to be 63.9% in the 5 mg/day treatment.[1] The RBC AChE inhibition serves as a convenient surrogate marker, per se, but it is not a valid marker for the central AChE inhibition process because inhibition in different tissues is a consequence of specific pharmacokinetics and pharmacodynamics that determine donepezil's accessibility to regional AChE. Currently, no peripheral surrogate marker has a valid one-to-one relationship with regional AChE inhibition in the brain.[12] Therefore, we did not perform RBC AChE assays.

There are several cholinesterase (ChE) inhibitors or the derivatives, such as methyl-tacrine,[28,29] physostigmine,[30] donepezil,[31] CP126 998, and 2-fluoro-CP118 954 labeled with ^{11}C or ^{18}F[32] for measurement of AChE binding sites. Most of them are not selective for AChE and have a considerable amount of non-AChE binding.[33] [^{11}C]donepezil has been applied to measure the effect of donepezil on AChE,[31] but it has high affinity for sigma receptor in addition to the AChE binding site.[34] The kinetic analysis of most radiolabeled ChE inhibitors, such as [^{11}C]donepezil, yields the distribution volume, which is the ratio between the brain concentration and plasma concentration of radioligands at equilibrium state.

In contrast, [^{11}C]MP4A and [^{11}C]MP4P are acetylcholine analogues and highly selective substrates for AChE, although [^{11}C]MP4P is somewhat less selective for AChE than [^{11}C]MP4A. Thus, the kinetic analysis of these radiolabeled acetylcholine analogue substrates allows measurement of AChE activity itself but not of the binding sites. These radiolabeled substrates enter the brain and are metabolized highly selectively by AChE. Carbon-11 labeled metabolites are practically irreversibly trapped in the brain and washed out very slowly, whereas unmetabolized substrates come out as the plasma concentration of radiolabeled substrates decreases. Thus, the kinetics of these radiolabeled substrates can be simplified as irreversible tracers, and the kinetic analysis of these radiolabeled substrates produces the k_3 value, an index of AChE activity. Therefore, the effect of donepezil on AChE may be more accurately measured with [^{11}C]MP4A or [^{11}C]MP4P than with the radiolabeled ChE inhibitors. [^{11}C]MP4A and [^{11}C]MP4P have been shown to be useful for the clinical study of dementias and therapeutic monitoring of the effects of cholinesterase inhibitors with reliable reproducibility. We previously assessed the reproducibility of [^{11}C]MP4A PET technique in five healthy controls and found that cortical k_3 values were nonsignificantly changed by −4 ± 10% in the second PET study without donepezil when compared with the baseline study.[13] Likewise, Kuhl et al[12] showed that PET measures of cerebral k_3 values repeated after 2-month intervals differed only by 6%. The level of precision is suitable for test-retest determination of inhibition effects.

In the present study using [^{11}C]MP4A PET, donepezil reduced k_3 values, an index of AChE activity, in the cortex by 34.6 ± 7.3% ($P < 0.0001$) compared with the baseline activity, confirming our preliminary study, which showed that donepezil reduced AChE activity in the cerebral cortex by 39 ± 5%.[13] Kuhl et al[12] investigated the effects of donepezil on AChE activity using [^{11}C]MP4P (PMP) and PET with arterial blood sampling and showed that cerebral cortical inhibition in 6 patients with AD after 5 and 10 mg of donepezil treatment averaged 26 ± 12% and 27 ± 11%, respectively. Bohnen et al[15] also investigated the effects of donepezil (10 mg/day, on cortical AChE activity using [^{11}C]MP4P and analyzed the data using the shape analysis without the plasma input function. The results showed that the average cortical (temporal, parietal, and dorsolateral prefrontal) donepezil-induced AChE inhibition after 10 mg of donepezil treatment in 14 patients with AD was 19.1 ± 9.4% compared with the baseline activity. Because donepezil is a highly selective inhibitor of AChE but not an inhibitor of butyrylcholinesterase, the lower inhibition rates by donepezil in these studies with [^{11}C]MP4P PET could be attributed to lower selectivity of MP4P for AChE than that of MP4A for AChE. The selectivity of MP4A and MP4P for AChE in human cerebral cortex was estimated to be 94% and 86%, respectively, in a postmortem brain study.[35]

Using [^{11}C]MP4A PET, Kaasinen et al[14] performed a study on 6 patients with AD and reported the regional difference in the inhibition rates in the cerebral cortex induced by donepezil. The treatment with 10 mg of donepezil reduced the AChE activity in the AD brain by 39% in the frontal cortex, 29% in the temporal cortex, and 28% in the parietal cortex. The degree of AChE inhibition was significantly greater in the frontal cortex than in the parietal and temporal cortices. Kaasinen et al[14] attributed the regional difference in AChE inhibition to the more severe reduction of AChE activity in the temporal cortex than in the frontal cortex at baseline in patients with AD. This study as well as the study of Kuhl et al[12] showed that the inhibitory effect of donepezil on AChE activity was uniform across the cerebral cortex.

According to the 24-week measurements of RBC AChE inhibition in the 5-mg/day donepezil treatment, the pharmacodynamics of donepezil was stable after 6 weeks of treatment.[36] The elimination of donepezil from the plasma was relatively slow at the 4.0- and 6.0-mg dose levels with an apparent half-life of approximately 80 hours.[25] The steady-state plasma concentration of donepezil after oral doses of 5 mg/day was reported as 29.6 ng/mL after 12 weeks of treatment in patients with AD[1] and 26.4 ± 3.9 ng/ml after 28 days of administration in healthy subjects.[37] The plasma concentration of donepezil in patients with AD in the present study (28.9 ng/mL) was almost the same in these previous studies.

To the best of our knowledge, there has been no in vivo human study concerning the quantitative relationship between

the plasma donepezil concentration and brain AChE inhibition. Readers may notice that data point in Figure 1 only cover narrow range of the total curve, and the correlation coefficient between the plasma donepezil concentration and the AChE inhibition rate remained 0.50 (borderline significantly higher correlation). The insufficient correlation rate may be due to the restricted regimen. At the time of this study, the dosing regimen in Japan was strictly set at daily doses of 3 mg for 2 weeks, followed by 5 mg/day, whereas taking 10 mg daily was not approved for clinical use, although it is available now. A wider range of donepezil doses would have revealed a significant correlation between the plasma donepezil concentration and the AChE inhibition rate. Indeed in vitro human study[19] and in vivo monkey study[17] showed that the inhibitory effects of AChE inhibitors are dose dependent on AChE activity. This premise encouraged us to estimate the in vivo plasma IC_{50} of donepezil for brain AChE inhibition in patients with AD. Currently, no peripheral surrogate marker, such as RBC AChE, has a valid one-to-one relationship with regional AChE inhibition in the brain.[12] Once the in vivo plasma IC_{50} of donepezil is estimated, the AChE inhibition rates in the cerebral cortex can be determined from the plasma concentration of donepezil in each patient with AD at various doses subsequently. Therefore, the in vivo plasma IC_{50} of donepezil is worthy to be estimated despite of the high costs and efforts for PET studies.

The plasma IC_{50} value was estimated at 54 ng/mL (130 nM) in the present study. Using the reported protein-bound fraction (92.6%) of donepezil in healthy human plasma,[38] we could estimate the plasma IC_{50} of donepezil in the free fraction as 9.6 nM (130 nM × [100-92.6]%). Both Yamanishi et al[39] and Ogura et al,[40] using rat brain homogenate at a highly diluted condition where protein binding is considered to be negligible, reported in vitro IC_{50} of donepezil as 6.7 nM. We previously estimated in vivo plasma IC_{50} of donepezil to be 6.6 nM in free fraction in 5 rhesus monkeys using [^{11}C]MP4A PET.[17] The slight difference in plasma IC_{50} of donepezil between these animal studies and the present human study could be attributed to the methodological differences in addition to species differences.

In summary, we estimated the in vivo plasma IC_{50} of donepezil for brain AChE inhibition in patients with AD to be 54 ng/mL (130 nM). Once the plasma IC_{50} of donepezil is determined, the brain AChE inhibition rate could be estimated from the plasma concentration of donepezil in each subject based on the plasma IC_{50}. Subsequently, the AChE inhibition rates in the cerebral cortex can be estimated from the plasma concentration of donepezil in each patient with AD at various doses. Now that the mean donepezil concentrations in the plasma at the dose of 5 mg/day remained 28.9 ng/mL, approximately half of the plasma IC_{50}, higher dose of donepezil might provide further benefits for patients with AD, if safe and tolerated. This technique can be also applied to measure the in vivo plasma IC_{50} of other ChE inhibitors, such as rivastigmine and galantamine, and may facilitate the determination of appropriate clinical dosages of cholinesterase inhibitors.

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