

Significant effects of cholinesterase inhibitors on tau pathology in the Alzheimer's disease continuum: An in vivo positron emission tomography study

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Abstract

Objectives: No prior study has assessed the effects of cholinesterase inhibitors (ChEIs) on tau pathology in the brains of patients with Alzheimer's disease (AD). Using positron emission tomography, this study aimed to investigate whether ChEIs reduce tau aggregation in amyloid-positive participants.

Methods: We analyzed datasets from the Alzheimer's Disease Neuroimaging Initiative and included amyloid-positive participants who had undergone baseline and 1- or 2-year follow-up AV-1451 positron emission tomography scans. We included participants treated with and without ChEIs (ChEIs group: $n = 15$, No-ChEIs group, $n = 45$). The annual change in tau aggregation was calculated as the difference in AV-1451- standardized uptake value ratio (SUVR) between the two scans divided by the time between scans. Group differences in annual AV-1451-SUVR change were examined.

Results: We found a significantly lower annual change in AV-1451-SUVR in the Braak 1/2 regions (entorhinal cortex and hippocampus) of participants taking ChEIs. Increased AV-1451-SUVR between the first and second examinations were observed in 22 of 45 participants not taking ChEIs and 2 of 15 participants taking ChEIs. Fisher's exact test showed a significant difference in the ratio of participants with increased AV-1451-SUVR between the groups.

Conclusions: The findings of this positron emission tomography study suggest that the administration of ChEIs has some neuroprotective effects in patients of the AD continuum, at least in the early stage of the disease progression. This in vivo effect may be mediated via tau, preventing amyloid β -induced neurotoxicity.

KEYWORDS

Alzheimer's disease, cholinesterase inhibitors, positron emission tomography, tau aggregation

Key points

- We used positron emission tomography to investigate whether cholinesterase inhibitors (ChEIs) reduce tau aggregation in amyloid-positive subjects

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (<http://adni.loni.usc.edu>). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete listing of ADNI investigators can be found at: https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

- Our data revealed significantly lower values of annual change in AV-1451-standardized uptake value ratio (SUVR) in the Braak 1/2 regions of subjects with ChEIs compared to those without ChEIs.
- We found a significantly lower ratio of subjects showing an increase in AV-1451-SUVR among subjects with ChEIs compared to those without ChEIs.
- This positron emission tomography study suggests that neuroprotective ChEI effects, possibly mediated via tau to prevent amyloid β -induced neurotoxicity, may exist in vivo in patients of the Alzheimer's disease continuum.

1 | INTRODUCTION

The cholinesterase inhibitors (ChEIs) donepezil, galantamine, and rivastigmine, as well as the *N*-methyl-D-aspartate-receptor antagonist memantine, are the only approved therapies for Alzheimer's disease (AD). ChEIs are mainly regarded as effective for symptomatic treatment of AD by increasing synaptic acetylcholine levels, thus improving the interaction among cholinergic neurons involved in cognitive function.¹ Together with this symptomatic mechanism, ChEIs may modify the disease through the protection of neurons. In previous studies, it has been suggested that ChEIs delay disease progression by inhibiting the progression of brain atrophy.²⁻⁴ Furthermore, a long-term follow-up study of donepezil treatment showed a decreased rate of decline in cognitive function.⁵

Recent animal and clinical studies have indicated that the dysfunction of tau is crucial for amyloid β ($A\beta$)-derived neurotoxicity, and its aberration and aggregation could be related to neurodegeneration and the progression of cognitive dysfunction. The clinical progression of AD is mainly related to the aggregation of neurofibrillary tangles, which are derived by the deposition of phosphorylated tau.⁶ Structural modifications of tau in AD are related to neuronal dysfunction and cell death. Glycogen synthase kinase-3 (GSK-3), a downstream target of the phosphoinositide-3-kinase-protein kinase B (Akt) pathway, plays a role in the death of neuronal cells.⁷ Recently, the hyperphosphorylation of tau via activation of GSK-3 has been proposed as one of the pathogenic mechanisms of AD.⁸ Decreased protein phosphatase 2A (PP2A) activity has also been suggested to play a role in tau hyperphosphorylation.⁹

It has been suggested that ChEIs inhibit $A\beta$ -induced neurotoxicity by suppressing GSK-3, in addition to upregulating nicotinic acetylcholine receptors.^{7,9} Donepezil was shown to decrease GSK-3 activity and has also been suggested to stimulate PP2A activity.⁹ This is important for the possible role of ChEIs as disease modifiers, as GSK-3 and PP2A activity regulates tau phosphorylation, which is essential for the formation of neurofibrillary tangles in AD. Thus, these previous studies support the notion that ChEIs modify the disease process of AD by reducing the aggregation of hyperphosphorylated tau. Recently, the availability of radiotracers that bind to tau (e.g., ¹⁸F-AV-1451) enabled in vivo imaging of tau pathology.¹⁰ However, no study has assessed the effect of ChEIs on tau pathology in the brains of patients with AD. In the present study, we

investigated whether ChEIs reduce the aggregation of tau in amyloid-positive participants in vivo using positron emission tomography (PET) imaging.

2 | MATERIALS AND METHODS

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (<http://adni.loni.usc.edu>). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance (MR) imaging, PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. For up-to-date information, see <http://www.adni-info.org>.

2.1 | Participants

Amyloid-positive participants of AD continuum diagnosed with MCI or AD, as well as cognitively normal (CN) participants, were included in this study based on the respective inclusion criteria: CN—participants without depression, MCI, and dementia and with mini-mental state examination (MMSE) scores between 24 and 30; MCI—participants with MMSE scores between 24 and 30, objective memory loss measured using the education-adjusted Wechsler Memory Scale Logical Memory II score, a clinical dementia rating of 0.5, absence of significant levels of impairment in other cognitive domains, essentially preserved activities of daily living, and an absence of dementia; and AD—MMSE scores less than 27, a clinical dementia rating of 0.5 or 1, and meeting the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria for probable AD.¹¹ We included ADNI-2 and ADNI-3 data, with no duplicate entries, from amyloid-positive participants aged 60–85 years (inclusive) with a florbetapir (AV-45) scan who had undergone baseline and 1- or 2-year follow-up AV-1451 scans. All participants were selected as amyloid-positive based on pre-established cutoffs (global florbetapir standardized uptake value ratio [SUVR] > 1.11).¹² Participants also underwent neuropsychological assessment at the time of the AV-1451 PET. We included participants who were taking ChEIs (ChEIs group: *n* = 15; MCI, *n* = 8 and AD, *n* = 7).

TABLE 1 Demographic characteristics and amyloid/tau PET SUVR values of amyloid-positive subjects with and without cholinesterase inhibitors (ChEIs)

Characteristic	Subjects without ChEIs (n = 45)	Subjects with ChEIs (n = 15)	Mann-Whitney U-test (Z) or Fisher's exact test (χ^2)	p Value	Effect size: Mann-Whitney U-test (r) or Fisher's exact test (ϕ)
CN, MCI, AD (n)	31, 14, 0	0, 8, 7	$\chi^2 = 37.6$	<0.001***	$\phi = 0.74^a$
Sex M/F	20/25	9/6	$\chi^2 = 1.09$	0.30	$\phi = 0.29$
Age (year)	76.3 \pm 6.2 [61–84]	74.2 \pm 7.5 [62–85]	Z = 0.90	0.37	r = 0.12
Education (year)	16.8 \pm 2.3 [12–20]	16.5 \pm 2.1 [14–20]	Z = 0.50	0.61	r = 0.06
MMSE					
1st Exam	28.2 \pm 1.9 [22–30]	24.1 \pm 4.3 [15–30]	Z = 3.51	<0.001***	r = 0.45 ^a
2nd Exam	28.0 \pm 2.5 [19–30]	21.6 \pm 5.8 [10–30]	Z = 4.23	<0.001***	r = 0.55 ^a
ADAS-cog					
1st Exam	10.5 \pm 4.1 [5.3–21.3]	20.4 \pm 7.9 [7.3–32.7]	Z = 4.21	<0.001***	r = 0.54 ^a
2nd Exam	11.0 \pm 4.4 [5.7–25.3]	22.4 \pm 11.3 [7.0–46.0]	Z = 4.09	<0.001***	r = 0.53 ^a
Cortical AV-45_SUVr	1.33 \pm 0.16 [1.12–1.71]	1.39 \pm 0.20 [1.14–1.72]	Z = 0.10	0.32	r = 0.04
Braak 1/2_AV-1451_SUVr (entorhinal cortex and hippocampus)					
1st Exam	1.44 \pm 0.29 [1.04–2.07]	1.77 \pm 0.37 [1.16–2.62]	Z = 2.95	0.003**	r = 0.38 ^a
2nd Exam	1.48 \pm 0.29 [0.98–2.43]	1.61 \pm 0.36 [1.02–2.51]	Z = 1.49	0.14	r = 0.02
Braak 3/4_AV-1451_SUVr (medial temporal and limbic regions)					
1st Exam	1.51 \pm 0.26 [1.15–2.55]	2.09 \pm 0.77 [1.32–3.66]	Z = 3.20	0.001**	r = 0.41 ^a
2nd Exam	1.58 \pm 0.31 [1.23–2.62]	2.04 \pm 0.80 [1.14–3.51]	Z = 1.90	0.06	r = 0.25
Braak 5/6_AV-1451_SUVr (neocortical regions)					
1st Exam	1.54 \pm 0.24 [1.18–2.73]	2.02 \pm 0.78 [1.40–4.36]	Z = 3.00	0.003**	r = 0.39 ^a
2nd Exam	1.59 \pm 0.27 [1.20–2.80]	2.02 \pm 0.80 [1.28–4.23]	Z = 1.70	0.09	r = 0.21

Note: Data are presented as the mean \pm SD [minimum–maximum].

Abbreviations: AD, Alzheimer's disease; ADAS-cog, Alzheimer's Disease Scale for cognitive subscale; CN, cognitively normal; F, female; M, male; MCI, mild cognitive impairment; MMSE, mini-mental state examination; PET, positron emission tomography; SD, standard deviation; SUVr, standardized uptake value ratio.

^ar And ϕ more than 0.3 were regarded as indicators of a substantial effect.

**p < 0.01.

***p < 0.001.

Of the 15 participants, 12, 2, and 1 were taking donepezil, rivastigmine, and galantamine, respectively. As control data, we included participants not taking antedementia, antidepressant, and/or other behavioral medication (No-ChEIs group, n = 45; CN, n = 31; MCI, n = 14). The no-treatment group did not include AD patients probably due to the prompt therapeutic intervention after the diagnosis of AD. The annual change in tau PET was calculated as the percentage of change in tau PET SUVR between scans [(2nd tau PET SUVR – 1st tau PET SUVR) \times 100/1st tau PET SUVR] divided by the time between scans (1 or 2 years). General data of the participants (age, sex, years of education, MMSE score, and Alzheimer's Disease Assessment Scale-cognitive subscale-11 [ADAS-cog] score) were extracted from the ADNI databases (Table 1).

2.2 | Standard protocol approvals, registrations, and patient consent

All participants provided written informed consent. The study was approved by the institutional review boards at all participating study sites.

2.3 | A β and tau PET analysis

¹⁸F-AV-1451 neuroimaging data obtained from the ADNI-2 and ADNI-3 databases were analyzed. AV-45 scans were collected within 1 year of the baseline AV-1451 scans. The acquisition and image

TABLE 2 Comparison of the annual change in tau PET SUVR between subjects with and without cholinesterase inhibitors (ChEIs)

Regions	Annual change in tau PET SUVR (%) ^a		Mann-Whitney U-test		
	Subjects without ChEIs (n = 45)	Subjects with ChEIs (n = 15)	Z	P	Effect size r
	Braak 1/2_AV-1451_SUVr (entorhinal cortex and hippocampus)	4.05 ± 20.3	-7.29 ± 14.8	2.59	0.01*
Braak 3/4_AV-1451_SUVr (medial temporal and limbic regions)	4.09 ± 13.0	-1.68 ± 10.7	1.05	0.29	0.14
Braak 5/6_AV-1451_SUVr (neocortical regions)	3.23 ± 11.2	0.42 ± 6.74	0.54	0.59	0.07

Note: Data are presented as the mean ± SD.

Abbreviations: PET, positron emission tomography; ROI, region of interest; SD, standard deviation; SUVR, standardized uptake value ratio.

^aThe annual change in tau PET was calculated for each individual and each of the three composite ROIs as the percentage of change in tau PET SUVR between scans [(2nd tau PET SUVR – 1st tau PET SUVR) × 100/1st tau PET SUVR] divided by the time between scans (1 or 2 years).

^br More than 0.3 was regarded as an indicator of a substantial effect.

* $p < 0.016$ (= 0.05/3).

preprocessing protocols used are publicly available on the ADNI database website (<http://adni.loni.usc.edu/>). Each subject's pre-processed PET image was coregistered using statistical parametric mapping (<https://www.fil.ion.ucl.ac.uk/spm/>) to that subject's MR image that was closest in time to the PET scan.

The AV-45 dataset represents the mean AV-45 uptake in cortical gray matter-weighted florbetapir in the region of interests (ROIs) for all participants. The ROIs included the bilateral frontal, anterior/posterior cingulate, lateral parietal, and lateral temporal cortices, as defined by the ADNI group. ROI-based AV-45 SUVRs were calculated with reference to the mean AV-45 uptake of the whole cerebellum. The details of the data processing method are described in "UC Berkeley- AV45 Analysis Methods (PDF)" (<https://ida.loni.usc.edu/pages/access/studyData.jsp>).

For the AV-1451 dataset, tracer retention was quantified in ROIs that anatomically approximated the pathological stages of tangle deposition delineated by Braak and Braak.¹³ The data were corrected for partial volume effects using the geometric transfer matrix approach.¹⁴ The weighted mean SUVR was calculated from three composite ROIs that corresponded to the anatomical definitions of Braak stages 1 and 2 (entorhinal cortex and hippocampus), 3 and 4 (medial temporal and limbic region), and 5 and 6 (neocortical region) with reference to the mean AV-1451 uptake of the inferior cerebellum. The details of the data processing method are described in "UC Berkeley-Flortaucipir (AV-1451) processing methods (PDF)" (<https://ida.loni.usc.edu/pages/access/studyData.jsp>).

2.4 | Statistics

The normality of the data of continuous variables was assessed using the Shapiro-Wilk test. Nonnormal data were analyzed using nonparametric methods. All demographic characteristics data of continuous variables showed a skewed distribution, and group differences across demographic characteristics were examined with nonparametric Mann-Whitney analyses. Fisher's exact test was used to examine differences in diagnosis and sex between groups

(Table 1). Effect sizes were calculated using $r = Z/\sqrt{N}$ for nonparametric Mann-Whitney analyses and ϕ for Fisher's exact test to estimate and compare the effects of statistical results of different sample sizes. "Small," "medium," and "large" effect sizes of r and ϕ are 0.1, 0.3, and 0.5, respectively. Values of r and ϕ more than 0.3 was regarded as a substantial effect in this study.

Annual changes in AV-1451-SUVr (%) for each individual and each of the three composite ROIs were calculated as the percentage of change in tau PET SUVR between scans divided by the time (1 or 2 years). Nonnormality was shown for the annual AV-1451-SUVr changes in all assessed regions, and group differences between participants with and without ChEIs were assessed by nonparametric Mann-Whitney analyses (Table 2).

To examine the effect of the disease stage, we compared the annual AV-1451-SUVr changes between CN and MCI in the participants without ChEIs, and MCI and AD in those with ChEIs, respectively. Nonnormality was shown for the annual AV-1451-SUVr changes in all assessed regions, and group differences were examined with nonparametric Mann-Whitney analyses (Table 3).

Of the 15 participants medicated with ChEIs, 10 patients also took memantine. To examine the combined effect of memantine on the annual changes in AV-1451-SUVr, we compared the group differences between participants medicated with ChEIs and those medicated with ChEIs and memantine. Furthermore, 5 of the 15 participants medicated with ChEIs also took antidepressant medications. To examine the combined effect of the antidepressants on the annual changes in AV-1451-SUVr, we compared the group differences between participants medicated with only ChEIs and those with ChEIs and antidepressants. The normality of annual changes in AV-1451-SUVr was not confirmed in any of the measured regions, and group differences were examined with nonparametric Mann-Whitney analyses (Table 4).

All statistical analyses were performed using SPSS for Windows 26.0 (IBM Japan). Statistical tests were two-tailed, and significance was defined as a p value less than 0.05/ n using Bonferroni correction (where n refers to the number of multiple comparisons).

TABLE 3 Comparison of the annual change in tau PET SUVR between diagnostic types in subjects with and without cholinesterase inhibitors (ChEIs)

Regions	Subjects without ChEIs				Subjects with ChEIs				Effect size:	
	CN (n = 31)	MCI (n = 14)	Mann-Whitney U-test (Z) or Fisher's exact test (χ^2)	Value p	MCI (n = 8)	AD (n = 7)	Mann-Whitney U-test (Z) or Fisher's exact test (χ^2)	Value p	Mann-Whitney U-test (Z) or Fisher's exact test (χ^2)	Value p
Demographic characteristics										
Sex M/F	14/17	6/8	$\chi^2 = 0.02$	0.89	3/5	6/1	$\chi^2 = 3.62$	0.06	$\phi = 0.49^a$	$\phi = 0.49^a$
Age (year)	76.0 ± 5.9 [62-84]	76.8 ± 7.1 [61-84]	Z = 0.81	0.42	74.3 ± 6.6 [65-85]	74.1 ± 9.0 [62-84]	Z = 0.17	0.87	r = 0.04	r = 0.04
Education (year)	16.9 ± 2.1 [13-20]	16.6 ± 3.0 [12-20]	Z = 0.20	0.84	17.3 ± 2.6 [14-20]	15.7 ± 1.0 [14-17]	Z = 0.89	0.40	r = 0.23	r = 0.23
MMSE	28.8 ± 1.6 [24-30]	26.1 ± 3.2 [19-30]	Z = 2.91	0.004*	25.8 ± 4.3 [18-30]	22.1 ± 3.7 [15-26]	Z = 1.81	0.07	r = 0.47 ^a	r = 0.47 ^a
ADAS-cog	9.8 ± 3.3 [6.0-21.3]	13.7 ± 5.3 [5.7-25.3]	Z = 2.63	0.009*	16.7 ± 6.9 [7.3-26.0]	24.5 ± 7.2 [15.3-32.7]	Z = 2.09	0.04*	r = 0.54 ^a	r = 0.54 ^a
Annual change in AV-1451-PET SUVR (%) ^b										
Braak 1/2_AV-1451_SUVR (entorhinal cortex and hippocampus)	5.38 ± 19.9	1.10 ± 21.6	Z = 0.81	0.42	-3.24 ± 4.47	-9.99 ± 18.6	Z = 0.41	0.46	r = 0.11	r = 0.11
Braak 3/4_AV-1451_SUVR (medial temporal and limbic regions)	3.99 ± 11.9	4.31 ± 15.6	Z = 0.66	0.51	-0.69 ± 6.17	-2.34 ± 13.3	Z = 0.81	0.86	r = 0.21	r = 0.21
Braak 5/6_AV-1451_SUVR (neocortical regions)	3.51 ± 11.8	2.62 ± 10.3	Z = 0.71	0.48	0.63 ± 6.24	0.28 ± 7.42	Z = 0.56	0.61	r = 0.14	r = 0.14

Note: Data are presented as the mean ± SD.

Abbreviations: AD, Alzheimer's disease; ADAS-cog, Alzheimer's Disease Scale for cognitive subscale; CN, cognitively normal; F, female; M, male; MCI, mild cognitive impairment; MMSE, mini-mental state examination; PET, positron emission tomography; ROI, region of interest; SD, standard deviation; SUVR, standardized uptake value ratio.

^ar And ϕ more than 0.3 were regarded as indicators of a substantial effect.

^bThe annual change in tau PET was calculated for each individual and each of the three composite ROIs as the percentage of change in tau PET SUVR between scans [(2nd tau PET SUVR - 1st tau PET SUVR) × 100/1st tau PET SUVR] divided by the time between scans (1 or 2 years).

*p < 0.05.

TABLE 4 Comparison of the demographics and annual changes in AV-1451-PET SUVR between subjects with and without memantine/antidepressants among subjects taking cholinesterase inhibitors (ChEIs)

	ChEIs without memantine (n = 5)		ChEIs with memantine (n = 10)		Mann-Whitney U-test (Z) or Fisher's exact test (χ^2)		Effect size: Mann-Whitney U-test (Z) or Fisher's exact test (χ^2)		Effect size: Mann-Whitney U-test (r) or Fisher's exact test (ϕ)	
Demographic characteristics										
Sex M/F	3/2	6/4	$\chi^2 = 0.00$	1.00	$\phi = 0.00$	7/3	2/3	$\chi^2 = 1.25$	0.26	$\phi = 0.29$
Age (year)	75.8 ± 9.4 [65-85]	73.4 ± 6.6 [62-81]	Z = 0.61	0.59	r = 0.16	76.7 ± 6.7 [62-85]	69.2 ± 7.1 [63-81]	Z = 1.59	0.13	r = 0.41 ^a
Education (year)	18.0 ± 2.0 [16-20]	15.8 ± 1.8 [14-20]	Z = 2.01	0.06	r = 0.52 ^a	16.3 ± 2.2 [14-20]	17.0 ± 2.0 [15-20]	Z = 0.76	0.51	r = 0.19
MMSE	27.6 ± 3.4 [22-30]	22.3 ± 3.7 [15-27]	Z = 2.28	0.02*	r = 0.59 ^a	24.1 ± 3.4 [18-29]	24.0 ± 6.3 [15-30]	Z = 0.31	0.77	r = 0.08
ADAS-cog	16.5 ± 6.6 [9.7-27.3]	22.3 ± 8.1 [7.3-32.7]	Z = 1.47	0.17	r = 0.38 ^a	20.2 ± 7.0 [7.3-29.3]	20.6 ± 10.4 [9.7-32.7]	Z = 0.12	0.95	r = 0.03
Annual change in AV-1451-PET SUVR (%) ^b										
Braak 1/2_AV-1451_SUVR (entorhinal cortex and hippocampus)	-7.81 ± 25.3	-7.03±7.27	Z = 1.59	0.13	r = 0.41 ^a	-7.68 ± 17.3	-6.49 ± 9.33	Z = 0.37	0.77	r = 0.10
Braak 3/4_AV-1451_SUVR (medial temporal and limbic region)	-7.12 ± 13.4	1.04 ± 8.63	Z = 1.59	0.13	r = 0.41 ^a	-3.74 ± 10.7	2.44 ± 10.6	Z = 0.37	0.77	r = 0.10
Braak 5/6_AV-1451_SUVR (neocortical regions)	1.25 ± 9.27	0.00 ± 5.65	Z = 0.12	0.95	r = 0.03	0.12 ± 7.99	1.02 ± 3.85	Z = 0.49	0.68	r = 0.13

Note: Data are presented as the mean ± SD.

Abbreviations: F, female; M, male; MMSE, mini-mental state examination; ADAS-cog, Alzheimer's Disease Scale for cognitive subscale; PET, positron emission tomography; ROI, region of interest; SD, standard deviation; SUVR, standardized uptake value ratio.

^ar And ϕ more than 0.3 were regarded as indicators of a substantial effect.

^bThe annual change in tau PET was calculated for each individual and each of the three composite ROIs as the percentage of change in tau PET SUVR between scans [(2nd tau PET SUVR - 1st tau PET SUVR) × 100/1st tau PET SUVR] divided by the time between scans (1 or 2 years).

*p < 0.05.

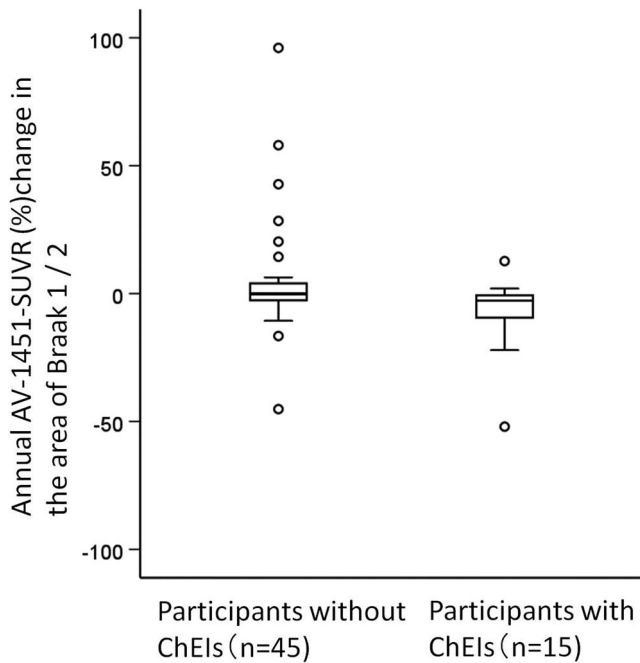


FIGURE 1 Box plot of the annual change in AV-1451 SUVR in the areas of Braak 1/2 (entorhinal cortex and hippocampus) in participants either taking or not taking cholinesterase inhibitors (ChEIs). There was a significant difference in the annual change in AV-1451-SUVR in the areas of Braak 1/2 (entorhinal cortex and hippocampus) between groups (Mann-Whitney *U*-test, $Z = 2.59$, $p = 0.01$). SUVR, standardized uptake value ratio

3 | RESULTS

Table 1 shows the demographics of the amyloid-positive participants with and without ChEI treatment. There was a significant difference in the percentage of diagnostic types between the two groups. There were no differences in sex, age, and years of education between the two groups. Lower cognitive function was observed in participants taking ChEIs in comparison to those not taking ChEIs. There were no between-group differences in cortical AV-45-SUVR. A significantly higher AV-1451-SUVR was observed at the first examination in participants taking ChEIs compared to those not taking ChEIs, but those differences between groups were not significant at the second examination. The effect sizes of the significant differences in Table 1 demonstrate a substantial effect of the groups.

Table 2 shows the comparison of the annual change in AV-1451-SUVR between participants taking ChEIs and those not taking ChEIs. We found a significantly lower annual change in AV-1451-SUVR in the regions of Braak 1/2 in participants taking ChEIs. The effect size demonstrates a substantial difference of the groups. A total of 22 of the 45 participants not taking ChEIs, but only 2 of the 15 participants taking ChEIs, showed an increase in AV-1451-SUVR between the first and second examinations (Figure 1). Fisher's exact test revealed a difference in the ratio of the participants with the increase in AV-1451-SUVR between groups ($\chi^2 = 5.93$, $p = 0.015$, $\phi = 0.31$).

Table 3 shows the comparison of the demographics and annual change in AV-1451-SUVR between diagnostic types in participants either taking or not taking ChEIs. There were no significant differences in the demographics except cognitive function between diagnostic types in participants taking ChEIs and those not taking ChEIs. The effect size of the significant differences in cognitive function demonstrate a substantial effect of the groups. We found no significant annual change in AV-1451-SUVR between diagnostic types in participants taking ChEIs and those not taking ChEIs.

Table 4 shows the comparison of the demographics and annual change in AV-1451-SUVR between participants taking memantine/antidepressants and ChEIs and those taking only ChEIs. We found medium-to-large effect sizes of group differences in education, MMSE, and ADAS-cog score, as well as AV-1451-SUVR in the areas of Braak 1/2 and 3/4, between participants taking memantine and ChEIs and those taking only ChEIs, although these differences did not reach statistical significance except for the MMSE score. Regarding AV-1451-SUVR in the areas of Braak 1/2 and 3/4, those taking only ChEIs presented lower average values. Apart from age, there were no significant or substantial differences in demographics and annual changes in AV-1451-SUVR in any of the comparisons between participants taking antidepressants and ChEIs and those taking only ChEIs.

4 | DISCUSSION

The main purpose of this study was to investigate whether ChEIs have neuroprotective effects by reducing the aggregation of tau in amyloid-positive participants. To the best of our knowledge, this is the first in vivo PET study to show the significant effect of ChEIs on tau pathology in the AD continuum. Our findings revealed a significantly lower annual AV-1451-SUVR change in the areas of Braak 1/2 in amyloid-positive participants taking ChEIs. Fisher's exact test confirmed a significantly lower ratio of amyloid-positive participants with an increase in AV-1451-SUVR among participants taking ChEIs in comparison to those not taking them.

There was a significant difference in the percentage of diagnostic types between participants taking ChEIs and those not taking them, and our data show lower cognitive function in participants taking ChEIs. However, we found no significant and substantial annual change in AV-1451-SUVR between diagnostic types; therefore, the stage of the disease process is not considered to be the main factor determining the results above.

Of the 15 participants with ChEI medication, 10 and 5 also took memantine and antidepressants, respectively. In the comparison of participants taking memantine and ChEIs and those taking only ChEIs, we found in the areas of Braak 1/2 and 3/4 medium effect sizes of the group difference of annual AV-1451-SUVR changes. However, the average annual AV-1451-SUVR change in these areas was lower in participants taking only ChEIs compared to those taking memantine and ChEIs. Furthermore, we found no significant or substantial differences in annual AV-1451-SUVR changes between

participants taking antidepressants and ChEIs and those taking only ChEIs. Based on these results, it is difficult to argue that these comedications are the main factor determining the annual decrease in AV-1451-SUVr in participants taking ChEIs.

Our findings indicate that ChEIs reduced the aggregation of tau in amyloid-positive participants in the regions of Braak 1/2 (entorhinal cortex and hippocampus), where neurodegeneration has been shown to be affected by tau pathology in the early stages of AD. We could not detect significant annual changes in AV-1451-SUVr in the areas of Braak 3–6. It is possible that in AD, the effect of ChEIs on tau aggregation is more prominent in the early stages of disease progression.

Although the symptomatic efficacy of ChEIs is assumed to take place through augmentation of cholinergic neurotransmission, previously published trials on ChEIs, including clinical or preclinical/in vitro studies, have indicated their possible disease-modifying or neuroprotective role through both cholinergic and noncholinergic mechanisms.^{7,9,15-23}

Regarding the neuroprotective role of ChEIs through their effect on phosphorylated tau aggregation, GSK-3 has been regarded as one of the essential enzymes regulating the pathogenic mechanisms of AD.²⁴ GSK-3 colocalizes with neurofibrillary tangles,²⁵ and its level of activity is increased in the brains of patients with AD.²⁶⁻²⁸ Furthermore, GSK-3 activation is suggested to be associated with the formation of paired helical filaments, neurite retraction, and neuronal death^{24,29} through phosphorylated tau both in vivo^{30,31} and in vitro^{31,32} at multiple sites in AD. Therefore, inhibition of GSK-3 activity might be effective for the treatment of AD.³³ In a previous study, the neuroprotective effects of donepezil were shown to be mediated by the inhibition of GSK-3, resulting in reduced phosphorylation of tau.⁷

Decreased PP2A activity also plays a role in the hyperphosphorylation of tau.⁹ PP2A is a major serine/threonine phosphatase that has essential roles in many biological processes.^{34,35} It can dephosphorylate tau at multiple sites.^{36,37} Evidence indicates the existence of mutual regulatory systems between kinases, including GSK-3 and phosphatases such as PP2A,³⁸⁻⁴⁰ and it is suggested that A β promotes tau phosphorylation by inhibiting PP2A activity.⁴¹ A study using an A β -derived neuronal toxicity model of AD showed that donepezil's neuroprotective effects against A β -induced neurotoxicity were mediated by the activation of PP2A.⁹ These previous studies support our finding that taking ChEIs decreases tau aggregation in the AD continuum. ChEIs may induce GSK-3 inhibition and PP2A activation, which would result in tau dephosphorylation and reduced aggregation of phosphorylated tau.

Our study has several limitations. First, we searched in ADNI for amyloid-positive participants of the AD continuum who underwent tau PET scans twice, and this necessary selection reduced the number of available participants. Second, we could not examine the effect of the *ApoE-4* allele on our results, as genotyping of *ApoE* was not performed for participants in the entire sample. Third, the annual change in AV-1451-SUVr showed a skewed distribution, and the group differences were examined with nonparametric Mann-Whitney analyses. Therefore, we could not apply parametric analysis with

demographic data, including diagnostic types and other medications as covariates. Fourth, only two and one of the 15 participants taking ChEIs were taking rivastigmine and galantamine, respectively. Due to the small number of these participants, we could not assess the difference in the effect on tau aggregation among the three ChEIs. Fifth, data regarding the ChEI administration period were only available for 4 of 15 participants (6 years for three participants and 3 years for one participant). Due to the small number of these participants, we could not assess the effect of the ChEI administration period on tau aggregation. Finally, investigations on additional effects of comedications such as antidepressants and memantine were based on a small sample ($n = 15$). It is difficult to conclusively confirm the absence of any specific effects of these comedications on tau aggregation in this study, and future studies with more participants are necessary.

Taken together, the results of our in vivo PET study suggest that the administration of ChEIs has some neuroprotective effects in patients of the AD continuum, at least in early disease stages. This in vivo effect may be tau-mediated, preventing A β -induced neurotoxicity. As this is a retrospective case-control study and may be affected by bias, future clinical cohort studies will be required to confirm the possibility of neuroprotective ChEI effects.

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CONFLICT OF INTERESTS

The authors have no conflict of interest to report. The sponsor had no role in the data analysis, interpretation of the data, or the preparation, review, or approval of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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