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High availability of the α7 nicotinic acetylcholine receptor in brains of individuals with mild cognitive impairment: A pilot study using ^{18}F-ASEM PET

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Abstract

Emerging evidence supports a hypothesized role of the α7-nicotinic acetylcholine receptor (α7-nAChR) in the pathophysiology of Alzheimer's disease (AD). ^{18}F-ASEM is a radioligand for estimating availability of the α7-nAChR in the brain *in vivo* with positron emission tomography (PET). **Methods:** In this cross-sectional study, 14 patients with mild cognitive impairment (MCI), a prodromal stage to dementia, and 17 cognitively intact, elderly controls completed ^{18}F-ASEM PET. For each participant, binding in each region of interest was estimated using Logan graphical analysis with a metabolite-corrected arterial input function. **Results:** Higher ^{18}F-ASEM binding was observed in MCI compared to controls across all regions, supporting higher availability of the α7-nAChR in MCI. ^{18}F-ASEM binding was not associated with verbal memory in this small MCI sample. **Conclusion:** These data support use of ^{18}F-ASEM PET to examine further the relationship between α7-nAChR availability and MCI.

Key Words

nAChR, cholinergic, ^{18}F-ASEM, dementia, positron emission tomography

INTRODUCTION

The α7-nicotinic acetylcholine receptor (α7-nAChR) may play a mechanistic role in the selective vulnerability of cholinergic cells to neurodegeneration in the earliest phases of Alzheimer's disease (AD) (*1*). The α7-nAChR binds soluble 42 amino-acid β-amyloid peptide (Aβ42) with picomolar affinity (*2*) and in AD, the Aβ42-α7-nAChR interaction may result in dysregulated signal transduction, compromised synaptic plasticity, and a toxic, selective effect on cholinergic cells of the basal forebrain (BF) and its projection sites (*3*). AD mouse models support upregulation of α7-nAChR expression in the presence of Aβ42 that promotes further pathology (*4*). In AD brain tissue, high levels of α7-nAChR co-localize with intracellular Aβ42 burden (*5*), and high α7-nAChR expression was found on BF (specifically nucleus basalis) cholinergic neurons (*6*). Relative to control tissue, a non-significant trend of higher BF α7-nAChR expression was found in cases of Mild Cognitive Impairment (MCI), a prodromal stage to dementia (*6*).

^{18}F-ASEM is a radioligand for estimating the availability of the α7-nAChR in the brain *in vivo* with positron emission tomography (PET) that showed high specific binding in receptor blockade studies in mice (*7*) and baboons (*8*). ^{18}F-ASEM PET has been used to study the α7-nAChR in healthy aging (*9*) and psychosis (*10*). In this pilot study we used ^{18}F-ASEM PET to test for higher *in vivo* availability of α7-nAChR in individuals with MCI compared to cognitively intact, healthy controls. Associations between regional ^{18}F-ASEM binding and memory were explored.

MATERIALS AND METHODS

Human Subjects

The Johns Hopkins Institutional Review Board approved this prospective study, which was conducted under an Investigational New Drug protocol approved by the US Food and Drug Administration. Participants (non-smokers over 65 years old) provided written informed consent. Participants completed a clinical assessment, magnetic resonance imaging (MRI), and comprehensive neuropsychological evaluation as in (*9*) that included the California Verbal Learning Test (CVLT) (*11*), which is sensitive to detecting memory impairment in MCI. All MCI participants had a clinical dementia rating global score (CDR) (*12*) of 0.5 with a sum of boxes (CDR-SOB) score not exceeding 2.5, and impaired memory on neuropsychological testing (at least one standard deviation below normal performance on a memory test). Control participants were in stable health with CDR = 0 (normal). Exclusion criteria included global CDR >0.5, decline in activities of daily living, nicotine use in the past six months, neurological condition other than MCI, substance abuse, current use of medication with potential to affect radiotracer binding (i.e. acetylcholinesterase inhibitors, anticholinergics), or contraindication to imaging.

The presence of an apolipoprotein E *(APOE)* ε4 allele is associated with cerebral amyloid burden (*13*). We classified participants as either an *APOE* ε4 carrier (presence of ≥1 ε4 allele) or an *APOE* ε4 non-carrier [see (*9*) for genotyping methods].

Brain Imaging

^{18}F-ASEM PET and MRI data were acquired and PMOD v3.7 (Zurich) was used for image processing as previously described (*9*). MRI volumetric segmentation was conducted with FreeSurfer software suite (http://surfer.nmr.mgh.harvard.edu/) or MRICloud (www.mricloud.org; for BF only). Regions of interest included cortical (frontal, parietal, temporal, occipital, cingulate, cerebellar) and subcortical (hippocampus, thalamus, striatum, BF) regions. Total intracranial volume (ICV) was also estimated using FreeSurfer for comparison of regional atrophy between the two groups while accounting for individual variability in head size. Regional volume normalized to ICV is referred to as regional volume ratio.

The kinetics of ^{18}F-ASEM were modeled using Logan graphical analysis (*14*) with a metabolite-corrected arterial input function obtained from 90 min dynamic data, generating regional ^{18}F-ASEM total distribution volume, V_T (*15*). To address effect of atrophy on ^{18}F-ASEM binding in the brains of this elderly study population, we report regional V_T values that were derived from PET data after partial volume correction (PVC) using the Muller-Gartner algorithm (*16*). V_T estimates from images without PVC were secondary binding outcomes.

Statistical Analysis

Group (control, MCI) differences in the ^{18}F-ASEM V_T were performed using a linear mixed effects regression model (MRM) to accommodate within-subject assessment of brain regions that are associated to different degrees. Primary predictors included group, an index variable for brain region, and the two-way interaction. Significance was set at $P < 0.05$ for this single MRM analysis. Pearson partial correlations were conducted to explore relationships within the MCI

group between ^{18}F-ASEM binding and performance on CVLT short and long delay free recall. Age was included in all models as a covariate since age may affect ^{18}F-ASEM binding (*9*). When group differences emerged, subsequent models were run with the adjustment for not only age but also race, sex, and *APOE* ε4 carrier status. Due to two primary memory outcomes, the threshold for significance was set as $P < 0.025$ (= 0.05/2) for examining these correlations.

RESULTS

Participants

Clinical characteristics, *APOE* ε4 carrier status, and relevant imaging parameters were similar between the control (N=17) and MCI (N=14) groups (Table 1). After age-adjustment, patients with MCI had higher scores than controls on the CDR-SOB (P=0.001) and lower scores on the two CVLT outcomes (each $P < 0.025$).

Imaging

In age-adjusted analyses, there was a near-significant group x region interaction ($F_{9,28}$=2.21, P=0.05) indicating that there were group differences in some but not all MRI-based segmentation regional volumes. Specifically, the MCI group had smaller hippocampal (P =0.001) and thalamic volumes (P =0.04) versus controls (Supplemental Table 1). The same pattern of differences was seen in regional volume ratios after age-adjustment.

The metabolism of ^{18}F-ASEM over the scan duration did not differ between groups at any time point with mean parent fraction at 10, 45, and 90 min post-injection of 69 ± 7%, 28 ± 9%, and 14 ± 5%, respectively among the MCI group, and 68 ± 6%, 29 ± 8%, and 15 ± 4%

respectively among the controls. In age-adjusted analyses using images after PVC, the MCI group had higher ^{18}F-ASEM V_T (estimated *M*=31.51; *SE*=1.14) versus controls (estimated *M*=27.23, *SE*=1.04; $F_{1,28}$=7.02, *P*=0.01; *d*=0.999; 95%CI 0.25-1.75), with the pattern of group differences similar across all regions ($F_{9,28}$ =1.37, *P* =0.25; Fig. 1, Supplemental Table 2). Re-running the model after further adjusting for race, sex, and *APOE* ε4 carrier status resulted in the same pattern of higher ^{18}F-ASEM V_T in MCI versus controls ($F_{1,24}$=11.03, *P* =0.003).

Among secondary ^{18}F-ASEM binding outcomes (Supplemental Table 3), a similar but non-significant binding pattern was observed using V_T derived from data without PVC and age-adjustment ($F_{1,28}$=3.81, *P* =0.06). After further adjusting for race, sex, and *APOE* ε4 carrier status, the group difference was significant ($F_{1,24}$=9.06, *P* =0.006).

Among patients with MCI, there were no associations between regional ^{18}F-ASEM V_T and CVLT performance (Supplemental Table 4).

DISCUSSION

Our ^{18}F-ASEM PET data are consistent with higher availability of the α7-nAChR in MCI compared to healthy controls across multiple brain regions. These pilot results align with postmortem studies reporting higher α7-nAChR levels in early stages of AD (*6*), and animal models of AD (*4,17*). If validated as a mechanistically-linked biomarker, high α7-nAChR availability may prove useful for earlier detection and therapeutic intervention in AD (*18,19*).

We acknowledge limitations in this study. First, V_T reflects specifically bound and nondisplaceable (non-specifically bound or free) radiotracer. Human ^{18}F-ASEM PET data do not support a brain region without the α7-nAChR that could be used as reference tissue for reporting the outcome of specifically bound (relative to nondisplaceable) ^{18}F-ASEM. This widespread α7-nAChR distribution in human brain is similar to that reported in rhesus monkeys, and differs from rodent brain (*20*). V_T was derived from images after PVC that adjusts the PET signal for regional brain atrophy seen in neurodegeneration. Consistent with published MCI work and supporting use of PVC, we saw smaller hippocampal and thalamic volumes in MCI compared to controls after age-adjustment. However, a risk of employing PVC is false elevation of regional V_T estimates due to overcompensation by the method. In this study, model analysis using V_T from images without PVC produced a similar, albeit non-significant pattern of higher binding in MCI compared to controls after age-adjustment, which achieved significance after further adjustment for other factors (sex, race, and *APOE* ε4 carrier status). Second, while these findings suggest a role of the α7-nAChR in MCI, the cross-sectional study design and lack of amyloid markers in our pilot population limit the ability to assess causality or specificity to AD-mediated MCI (as opposed to MCI from non-AD pathology). The lack of association between ^{18}F-ASEM V_T and verbal memory impairment in this study may be due to possible inclusion of MCI participants with non-AD pathology. Furthermore, here we focused on brain tissue, but the α7nAChR in cerebral vasculature may promote cerebral amyloid angiopathy (*21*) that is common in AD and linked to impaired memory. Complementary, animal studies that manipulate α7-nAChR availability or activity should be pursued to evaluate causal, mechanistic relationships between the α7-nAChR and AD pathophysiology. Future studies designed to test the

relationships between ^{18}F-ASEM binding and neuropsychological performance in larger clinical samples are needed.

CONCLUSION

Our ^{18}F-ASEM PET data support a higher availability of the α7-nAChR across several brain regions in individuals with MCI compared to healthy individuals.

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Figure 1

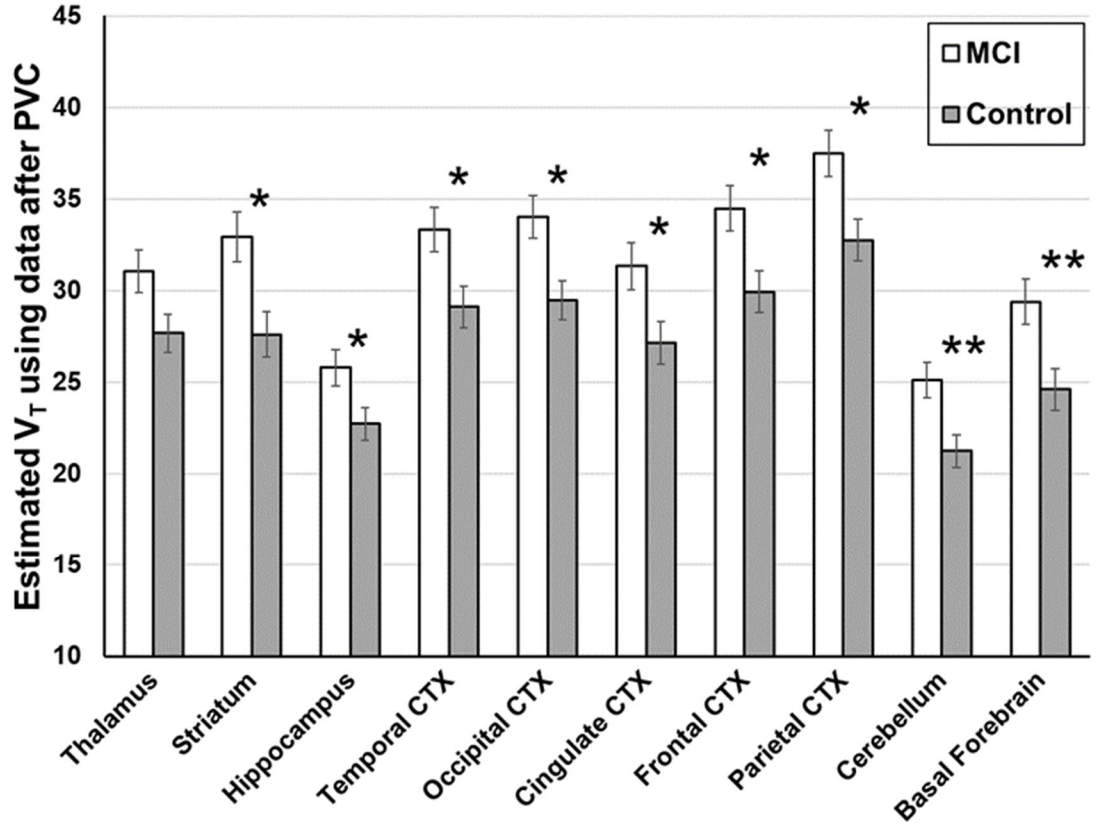

Figure 1. Higher ^{18}F-ASEM regional total distribution volume (V_T) values were found in 14 participants with mild cognitive impairment (MCI) compared to 17 cognitively intact healthy controls participants, after accounting for age. Boxplot diagram of ^{18}F-ASEM V_T derived from images after partial volume correction (PVC) across ten regions of interest from participants with MCI (white boxes) and control participants (gray boxes). A linear mixed effects regression model adjusting for age and brain region demonstrated higher V_T in MCI compared to controls. In secondary analyses, a regional difference for each region except thalamus was found between MCI and controls using individual linear regression models fit for the region of interest and controlling for age (*P <0.05; **P <0.01). These differences in individual regions did not remain significant after Bonferroni correction for the ten regions tested, which required P <0.005. V_T is in units of mL cm^{-3}.

Tables

Table 1. Clinical characteristics and PET parameters among healthy controls and patients with mild cognitive impairment (MCI).

	Control (N = 17) Mean ± SD	**MCI (N = 14)** Mean ± SD	***P***
Age (years)	76.4 ± 6.2	80.5 ± 5.5	0.06
Sex (male), n (%)	9 (53%)	7 (50%)	0.88
Race (AA; Cauc; Asian), n (%)	4 (24%); 12 (71%); 1 (6%)	7 (50%); 7(50%); 0 (0%)	0.24
Education (years)	17.1 ± 3.3	15.7 ± 4.3	0.35
Body Mass Index	26.5 ± 3.0	26.7 ± 3.2	0.92
Genetic Vulnerability			
APOE ε4 Carrier (yes), n (%)	3 (18%)	5 (36%)	0.28
Cognitive Performance*			
CDR-SOB	0.0 ± 0.1	1.1 ± 0.7	< 0.001
CVLT Short Delay Free Recall	11.2 ± 2.9	7.9 ± 4.0	0.02
CVLT Long Delay Free Recall	11.8 ± 2.7	7.6 ± 5.0	0.02
PET parameters			
Molar Activity (GBq/μmol)	3878.1 ± 2614.6	3327.1 ± 2133.8	0.52
Injected dose of radioactivity (MBq)	516.2 ± 22.2	517.2 ± 17.7	0.89
Injected mass (μg)	0.08 ± 0.06	0.11 ± 0.12	0.43

Abbreviations: AA, African American; Cauc, Caucasian; CDR-SOB, Clinical Dementia Rating Scale Sum of Boxes; CVLT, California Verbal Learning Test; SD, standard deviation. *P*-values from comparison using student's t test or chi-square test as appropriate except for comparison of cognitive performance* that were assessed using analysis of covariance with age as the covariate.