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Plasma acetylcholinesterase activity correlates with intracerebral β-amyloid load

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Abstract

Background—Previous studies have demonstrated alterations in the peripheral cholinergic system in Alzheimer's disease (AD), though results have been inconsistent and not linked to *in vivo* biomarkers of pathology. We examined the relationship between amyloid-beta (Aβ) plaques and plasma cholinesterase activity in a heterogeneous dementia population.

Methods—29 participants with clinical AD and 35 with non-AD diagnoses underwent positron emission tomography (PET) with the amyloid ligand [11C] PIB and plasma measurements of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity. Multi-linear regression was used to evaluate the relationship between AChE or BChE activity and PIB binding (adjusted for age, sex, apolipoprotein E4 and vascular risk), applying voxel-wise and region of interest (ROI) approaches. AChE activity was further adjusted for cholinesterase inhibitor (ChE-I) use. Global amyloid load was measured using a PIB Index, representing mean tracer binding in frontal, parietal, lateral temporal and cingulate cortex.

Results—AChE activity was correlated with PIB Index (β=0.39, p<0.001) and with regional PIB binding in frontal, temporal, parietal and occipital lobes, precuneus and posterior cingulate on both voxel-wise (p<0.001 uncorrected) and ROI (β=0.26-0.41, p<0.005) analysis. Correlations remained significant after covarying clinical diagnosis (β=0.42, p=0.001), and among participants naive to ChE-I (β=0.51, p=0.005). No correlation was found between BChE activity and PIB. Among AD participants, disease severity was not correlated with AChE, BChE or PIB Index.

Conclusion—AChE activity in plasma is correlated with brain Aβ load. Activation of the 'anti-inflammatory cholinergic pathway' may provide the link between Aβ plaques and peripheral cholinergic measures.

Introduction

Two of the main features of Alzheimer disease (AD) are amyloid-β (Aβ) plaques (1) and dysfunction of the cholinergic system (2, 3). Aβ plaque accumulation is thought to be a continuous process that takes place over 10-15 years and reaches a relative plateau before

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the evolution of cognitive impairment (4). In many (but probably not all) individuals, Aβ aggregation triggers a cascade of events that lead to network dysfunction,, neurodegeneration and ultimately to clinical dementia (5). Aβ aggregates, in particular soluble oligomers, are thought to have a direct neurotoxic effect (6). In addition, Aβ may induce neurodegeneration indirectly by initiating a pro-inflammatory cascade that results in the release of neurotoxic cytokines (7,8). The dysfunction and loss of basal forebrain cholinergic neurons and consequent decrease in acetylcholine (ACh) levels also contribute to cognitive impairment in AD (9). Cholinesterases, the enzymes that catalyze the hydrolysis of ACh, play a central role in the regulation of cholinergic neurotransmission. The predominant cholinesterases are acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Most studies report decreased AChE activity in the cerebrospinal fluid (CSF) of AD patients, compatible with the early loss of cholinergic neurons in AD. Reports regarding changes in BChE activity in AD are inconsistent (10-12). One study reported a negative correlation between BChE activity levels in the CSF and Aβ load *in vivo* (13). Increasing cholinergic activity in the central nervous system via cholinesterase inhibition is the current mainstay of the pharmacologic treatment of AD (14).

The inflammatory response triggered in the brain by Aβ deposition may affect peripheral cholinesterase (ChE) levels via the peripheral cholinergic system. Inflammatory stimuli induce the vagus nerve to increase ACh release, which in turn acts as an anti-inflammatory agent (15). Therefore, measuring peripheral ChE activity may inform us about the presence of Aβ plaques and could potentially serve as a serologic biomarker for AD pathology.

The objective of this study was to examine the relationship between serum ChE activity and brain Aβ load, as measured in vivo by positron emission tomography with the Aβ-specific tracer Pittsburgh Compound-B (PIB-PET) (16). We hypothesized that increased brain Aβ load would be associated with increased serum ChE activity, reflecting activation of the cholinergic anti-inflammatory pathway by the neuro-inflammation associated with Aβ deposition.

1. Methods

1.1. Participant selection and characterization

Sixty four participants were recruited from dementia research cohorts followed at the University of California San Francisco Memory and Aging Center. The clinical evaluation included a history and physical examination by a neurologist, a structured caregiver interview by a nurse, and a battery of neuropsychological tests (17). Global cognition was assessed using the Mini Mental State Examination (MMSE), and functional impairment was measured via the Clinical Dementia Rating (CDR). Clinical diagnosis was assigned by consensus at a multi-disciplinary conference. Participants with significant co-morbid medical, psychiatric illness or cerebrovascular disease were not eligible for the study. A heterogeneous dementia population was studied in order to include individuals with a broad continuum of brain Aβ load. Participants' medical records were reviewed to assess for vascular risk factors that can impact systemic inflammation and thus impact peripheral ChE activity (15, 18). A 'Vascular Index' was calculated by adding 1 point each for hypertension, hypercholesterolemia, diabetes mellitus and recent (<10 years prior) tobacco use, and 2 points for active smoking and cardiovascular disease (modified from Framingham criteria (19) to integrate available data on participants' past medical history).

1.2. Plasma measurements

Plasma from participants was collected between March 2006 and November 2008. Plasma cholinesterase catalytic activity was measured by a spectrophotometric assay.

Acetylthiocholine (ATCh, Sigma, 1 mM) hydrolysis rates were measured by placing 10 μL 1:20 diluted plasma in microtiter plate wells, after 20 min pre-incubation with or without 50uM iso-OMPA (Sigma), a specific BChE inhibitor. Readings at OD405 nm were repeated at 2-min intervals for 10 min. Non-enzymatic breakdown of substrate was subtracted from the total rate of hydrolysis. Enzyme activities were calculated using the OD_{405} for 5-thio-2-nitrobenzoate, 13,600 M/cm..

1.3. PIB-PET

1.3.1. PIB-PET acquisition—All participants underwent PIB-PET imaging at Lawrence Berkeley National Laboratory between April 2005 and February 2009. Average time between plasma collection and PIB was 0.69 ±0.70 years (range 0-2.87). [^{11}C]PIB was synthesized at the Lawrence Berkeley National Laboratory's Biomedical Isotope Facility. PET scans were performed with a Siemens ECAT EXACT HR PET scanner in 3D acquisition mode. 15 mCi of PIB were injected as a bolus into an antecubital vein and dynamic acquisition frames were obtained for 90 min, as previously described (20). PET data were reconstructed using an ordered subset expectation maximization algorithm with weighted attenuation. Images were smoothed with a 4 mm Gaussian kernel with scatter correction. All images were evaluated before analysis for participant motion and adequacy of statistical counts.

1.3.2. PIB-PET analysis—Image processing and analysis was performed using Statistical Parametric Mapping (SPM) software (http://www.fil.ion.ucl.ac.uk/spm). To allow for inter-subject comparisons, voxel-wise distribution volume ratios (DVR) were calculated using Logan graphical analysis, with the cerebellum time-activity curve used as a reference tissue input function (t = 35–90 min).

1.3.3. Voxel-wise analysis—Individual PIB volumes were spatially normalized to Montreal Neurological Institute (MNI) space using SPM5. Mean PIB images created during frame realignment were normalized to the SPM PET template, and the normalization parameters were then applied to the PIB DVR volumes. Spatially-normalized images were smoothed with a 12 mm kernel. Voxel-wise multi-linear regression was performed examining the relationship between PIB binding and AChE activity, adjusting for age, sex, apolipoprotein E4 (ApoE4) allele status (carrier or non- carrier), Vascular Index and cholinesterase inhibitor (ChE-I) treatment. A similar model was used to test the relationship between PIB and BChE activity with two exceptions: participants taking rivastigmine (a non-selective ChE-I that affects both AChE and BChE) were excluded, and ChE-I use was not included as a covariate since donepezil and galantamine do not have a biological effect on BChE). To allow broad visualization of the data, results were displayed on a template brain as T-maps threshold at $p<0.001$ uncorrected for multiple comparisons.

1.3.4. Region of interest (ROI) analysis—PIB DVR values were extracted in normalized space from regions of interest derived from the Automated Anatomic Labeling Atlas (21). The ROIs were: frontal, parietal, temporal and occipital cortex, hippocampus, precuneus and posterior cingulate cortex. In order to exclude white matter and cerebrospinal fluid, automated regions of interest were masked by the individual subject's gray matter segmented images. A PIB index, representing the mean DVR throughout frontal, parietal, lateral temporal and cingulate cortices was used as a measure of global amyloid burden.

1.4. Statistical analysis

Multi linear regression was used to evaluate the relationship between PIB binding (independent variable) and AChE activity (dependent variable) across all subjects, and then separately among AD and non-AD participants. The following covariates were included in

the model: age, sex, ApoE4 status (carrier or non- carrier), Vascular Index and ChE-I treatment (separately for donepezil, rivastigmine and galantamine, as each ChE-I may have a different effect on AChE activity level (22)). Due to the small sample size, we repeated the analysis with no covariates, using PIB Index as a single independent variable. For the evaluation of the relationship between PIB binding (independent variable) and BChE activity (dependent variable) the model included: age, sex, ApoE4 allele status and Vascular Index. As in the voxel-wise analysis, subjects using rivastigmine were excluded and ChE-I treatment was not included as a covariate. Again we repeated the analysis with no covariates, using PIB Index as a single independent variable. The model then evaluated separately the relationship among AD and non-AD participants. Among participants with clinical AD, the correlation between disease severity and cholinesterase (AChE and BChE) activity was tested using MMSE or CDR sum-of boxes (CDR-sb) as measures of disease severity. Additional covariates included in the model were: age, sex, ApoE4 allele status (carrier or non- carrier), Vascular Index and ChE-I treatment. The relationships between PIB Index (dependent variable) and MMSE/ CDR-sb (independent variables) were assessed using multi-linear regression adjusting for age, sex, education and ApoE4 allele status (carrier or non- carrier). All analyses were conducted in SPSS (version 18.0).

The study was approved by the University of California Berkeley, University of California San Francisco and Lawrence Berkeley National Laboratory Institutional Review Boards for Human Research.

2. Results

2.1. Participant characteristics

Our final cohort consisted of 64 individuals (Table 1). 29 participants had a clinical diagnosis of AD (including 1 Lewy-body variant), and 35 had a non-AD diagnosis (27 frontotemporal lobar degeneration, 3 dementia of unknown cause, and 5 non-demented participants). The cohort was characterized by male preponderance and high education. 27 participants had either no cognitive impairment (CDR=0), or mild cognitive impairment (CDR=0.5) and 36 participants met criteria for dementia (CDR≥1). One non AD participant lacks a CDR score. 32/64 participants were taking ChE-I: donepezil (n=21) rivastigmine (n=5) or galantamine (n=6).

2.2. PIB: voxel-wise correlations

AChE activity correlated with PIB binding in diffuse cortical regions including bilateral middle frontal gyrus, precuneus, middle and posterior cingulate gyrus, inferior parietal lobule, superior greater than middle and inferior temporal gyrus, superior and middle occipital gyrus, calcarine cortex and lingual gyrus ($p<0.001$ uncorrected, Fig. 1). Additional correlations were found in right anterior cingulate, and right superior and inferior frontal gyrus. No correlation was found between BChE activity and PIB binding at $p<0.001$ uncorrected (Fig. 1).

2.3. PIB: region of interest correlations

AChE activity was positively correlated with PIB DVR values throughout all ROIs ($p<0.01$) except hippocampus (Table 3). Global amyloid burden, measured by PIB index, was positively correlated with AChE activity ($\beta=0.39$, $p<0.001$) (Table 2, Fig. 2a), with similar results when using PIB Index as a single independent variable ($\beta=0.395$, $p=0.001$). A model in which clinical diagnosis (AD and non-AD) was added as an independent variable (to account for the potential confounding effect of clinical AD on both PIB and AChE activity) yielded similar results ($\beta=0.42$, $p=0.001$). On evaluation of non-AD participants the correlation remained positively significant ($\beta=0.39$, $p=0.01$) (Fig. 2c), while among AD

participants the correlation was marginally significant (β=0.30, p=0.08) (Fig. 2b). The analysis was repeated including only individuals who were naïve to ChE-I treatment (n=32), to assure that the correlation between PIB Index and AChE activity was not confounded by ChE-I use among AD subjects, who are also likely to show high PIB binding. AChE remained positively correlated with PIB index (β=0.51, p=0.006) (Fig. 2d). BChE activity was not correlated with PIB DVR values in any of the ROIs (Table 3) or with PIB index (β=−0.123, p=0.383) (Table 2, Fig. 2e). Using the model with PIB Index as a single independent variable yielded similar results (β=−0.186, p=0.162). BChE correlated with sex (F>M) (β=0.29, p=0.03), compatible with previous observations (23), but not with any other variable in the model (Table 2).

2.4. AChE activity and ChE-I use

Use of donepezil, a rapidly-reversible ChE-I, correlated positively with AChE activity in the serum (β=0.61, p<0.001). There was a trend for a negative correlation between AChE activity levels and use of rivastigmine, a pseudo-irreversible ChE-I (β=−0.20, p<0.06). Use of galantamine, a rapidly-reversible ChE-I that also acts as a weak nicotinic receptor agonist (24), showed no correlation with AChE activity levels (Table 2).

2.5. Disease severity and cholinesterase activity among participants with clinical AD

Among participants with clinical AD, neither AChE nor BChE activity were correlated with disease severity, as measured by MMSE (β=0.13, p=0.40 and β=0.12, p=0.36) or CDR-sb (β=−0.27, p=0.05 and β=−0.02, p=0.86). In addition, no correlation was found between PIB Index and disease severity as measured by MMSE (β=0.02, p=0.94) and CDR-sb (β=−0.14, p=0.51).

3. Discussion

This is, to our knowledge, the first study to examine the relationship between plasma cholinesterase activity and brain Aβ plaques measured *in vivo* by PIB-PET. As hypothesized, we found a positive correlation between AChE activity and PIB binding. As PIB is a specific marker of Aβ fibrillar deposits which can be found both in AD and non-AD subjects, we correlated PIB binding and ChE activity across all subjects. This correlation remained significant after adjusting for the potential confounding effects of clinical diagnosis (by separately examining AD vs. non-AD groups, or by adding diagnostic group as a covariate) and ChE treatment. These findings suggest that deposition of Aβ in the brain is reflected in peripheral cholinergic activity. However, whether increased cholinergic activity in the periphery drives or is caused by Aβ deposition is unclear.

The link between brain Aβ and the peripheral cholinergic activity may be mediated by the ‘cholinergic anti-inflammatory pathway’ (Fig. 3). Minor signs of neuroinflammation can be found in the normal aging brain and are exacerbated by AD (7, 25). Aβ aggregates play a pivotal role as inducers of neuroinflammation via activation of microglial cells, which in turn release neurotoxic cytokines (8, 26). While pro-inflammatory cytokines exacerbate inflammation, other cytokines act to restrain it by activating anti-inflammatory responses. One of the rapid anti-inflammatory responses is the ‘cholinergic anti-inflammatory pathway’, which alleviates inflammation by increased release of ACh to the periphery via the vagus nerve (27, 28). ACh then acts as an anti-inflammatory agent by deactivating tissue macrophages (29, 30) and glial cells in the brain (31). Hyper-activation of the ‘cholinergic anti-inflammatory pathway’ increases ACh release and therefore leads to an increase in AChE activity, which then acts to dampen the excessive cholinergic signal (32, 33). Activation of the cholinergic anti-inflammatory pathway by Aβ-mediated processes thus

provides a plausible mechanism for the observed positive correlation between PIB binding and peripheral ChE activity.

Since cytokines cross the blood brain barrier (34), it is also possible that activation of the 'cholinergic anti-inflammatory pathway' is mediated by peripheral pro-inflammatory cytokines. Studies suggest that systemic inflammation might be associated with increased risk of developing AD (35-37). Pro-inflammatory cytokines have been shown to enhance Aβ deposition through increased expression of BACE1, which mediates the initial step in the cleavage of amyloid precursor protein to Aβ, and suppression of Aβ clearance (38). Therefore, exacerbation of Aβ deposition due to increased peripheral pro-inflammatory cytokines might provide another plausible explanation of our findings.

The association between the cholinergic and inflammatory pathways is quite complex. Recent studies have shown that the brain's cholinergic signaling activates a peripheral blockade over inflammation (39). This in turn limits the penetration of pro-inflammatory cytokines into the brain and reduces the production of AChE in cholinergic neurons. Within the brain, as well as in nucleated blood cells, AChE is by far the major cholinesterase, and changes in one of these enzymes do not necessarily predict similar changes in the other. An example is post-stroke conditions, where plasma AChE levels decline whereas BChE levels increase (40)

Clinical decline in AD is correlated with decreased AChE activity in the cerebral cortex (41, 42), but not with amyloid burden, which has either reached a plateau in early stages of AD or continues to progress very slowly (43). Consistent with previous studies, we found no correlation between PIB load and disease severity (44, 45). The lack of correlation between disease severity and plasma cholinesterase activity in AD patients in our cohort strengthens our hypothesis that acetylcholinesterase activity in the plasma is a reflection of amyloid burden rather than central cholinergic function.

We also found a positive relationship between ChE-I treatment with donepezil and plasma AChE activity. This relationship is perhaps counter-intuitive, given that the goal of ChE-I treatment is to dampen AChE activity and thus enhance cholinergic transmission. However, our results are consistent with previous studies which reported increased AChE activity in plasma and CSF among AD patients who were treated with donepezil (22, 46-48). It is possible that suppression of ChE activity in AD patients is transient, and that treatment may lead to a "rebound" of activity via compensatory mechanisms (49), perhaps explaining the apparently transient benefit of ChE-I treatment (50). Rivastigmine has a pseudo-irreversible inhibitory effect on acetyl- and butyrylcholinesterase and also was shown to have a potential neuroprotective effect, by altering APP (Aβ precursor) and Aβ secretions (51). The negative trend between rivastigmine use and AChE is compatible with previous studies (46, 52). The lack of relationship between galantamine and AChE activity can be attributed to its weak AChE inhibitory activity (45). However, due to the small sample size of ChE-I treated individuals in this study (particularly with galantamine and rivastigmine), these observations should be considered preliminary. Further studies are needed to examine the biological effects of ChE-I treatment over time in AD.

Our study has limitations. No direct inflammatory markers were measured and thus we could not directly test the hypothesis that Aβ deposition and peripheral ChE activity are linked by activation of the cholinergic anti-inflammatory pathway. Our cohort is primarily composed of patients with dementia, and most have some degree of neurodegeneration (related to either AD or non-AD pathology). Thus, it is conceivable that inflammation is driven by brain pathology other than Aβ plaques. We do not have biomarkers of non-Aβ pathology in this cohort (e.g. CSF tau levels). Structural MRIs were performed on a variety

of different MRI scanners and field strengths (1.5T, 3T and 4T), precluding a primary analysis of the relationship between atrophy/neurodegeneration and ChE activity. We measured total AChE activity and thus cannot distinguish the relative contributions of AChE subtypes, which are known to have different effects on Aβ accumulation. Finally, though our heterogeneous dementia population included a spectrum of Aβ pathology, patients with clinical AD were highly likely to have high PIB (93%) and be treated with ChE-I (69%), potentially confounding our analysis. Reassuringly, the correlation between PIB and ChE activity remained significant when adjusting for ChE-I use and clinical AD and in the subset of patients naïve to ChE-I treatment, suggesting it was not spuriously driven by ChE-I treatment in AD patients.

Future studies investigating the in vivo relationship between Aβ and cholinergic activity should incorporate measurements of inflammatory markers in the serum and in the CSF, to establish the connection between Aβ and the 'cholinergic anti-inflammatory pathway'. As CSF $A\beta_{1\text{-}42}$ and PIB-PET was shown in previous studies are highly correlated (53), studying the correlation between Aβ levels in the CSF and plasma AChE would reinforce the correlation we found between brain Aβ plaques and plasma AChE, and its potential role as a biomarker for AD. It would also be informative to study the relationship between peripheral ChE activity and the integrity of cholinergic nuclei in the basal forebrain, as well as the relationship between ChE activity and more direct measures of neurodegeneration in AD, such as temporoparietal hypometabolism and cortical and hippocampal atrophy.

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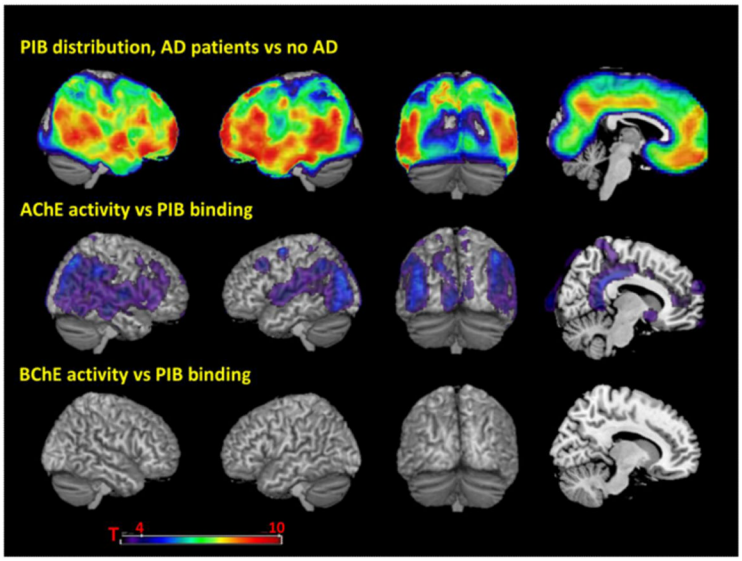

Figure 1.

Top row, patterns of PEB binding in Alzheimer's disease (AD) compared to non-AD participants. *Middle row*, voxel-wise multi-linear regression of PIB binding and AChE activity, adjusting for age. sex, apolipoprotem E4 (ApoE4) allele status (carrier or non-carrier). Vascular Index and cholinesterase inhibitor (C'hE-I) treatment. *Bottom row*, voxel-wise multi-linear regression of PIB binding and BC'hE activity, adjusting for age. sex. apolipoprotem (ApoE4) allele status (carrier or non- carrier), excluding participants taking rivastigmine (a non-selective ChE-I that affects both AChE and BChE). All results are presented at a threshold of $P < 0.001$. uncorrected for multiple comparisons.

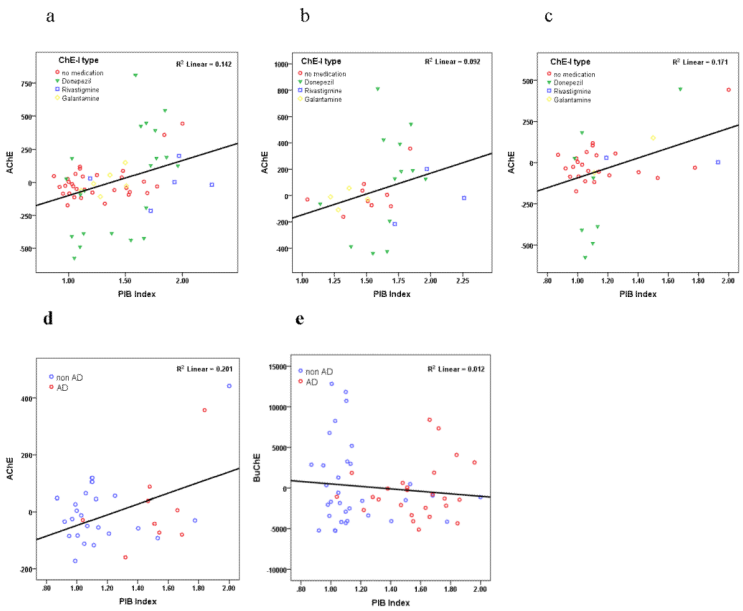

Figure 2.

Partial regression plots show the relationships between PIB Index and Cholinesterase activity, (a) AChE vs PIB Index among all participants, with AChE-I symbols (b) AChE vs PIB Index among AD participants (c) AChE vs PIB Index among non AD participants (d) AChE vs PIB Index among participants naïve to AChE-I treatment (e) BChE vs PIB Index. Residuals are plotted for each participant to adjust for the effects of age, gender, Vascular Index, ApoE4 status and ChE-I treatment.

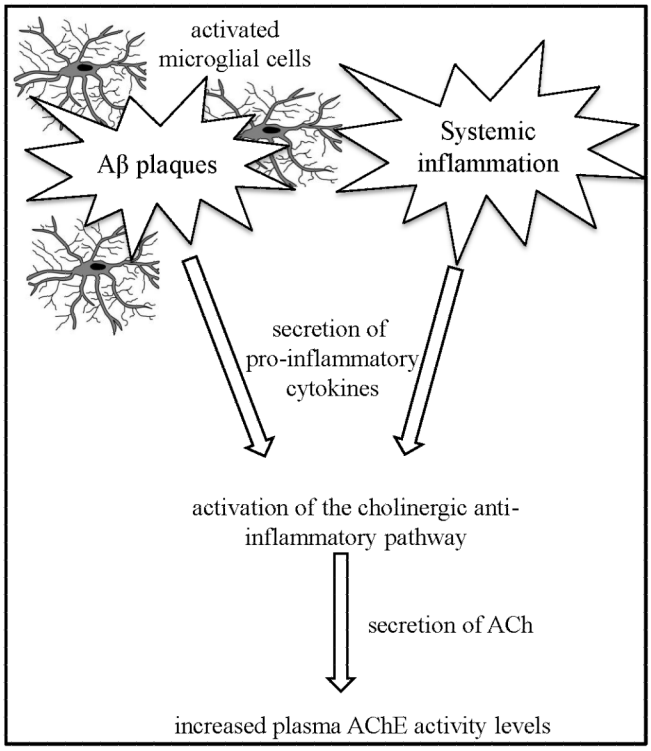

Figure 3.

Activation of the cholinergic anti-inflammatory pathway - Microglial cells activated by Aβ plaques/ongoing systemic inflammation increase the secretion of proinflammatory cytokines. The cytokines activate the 'cholinergic anti-inflammatory pathway' which increases ACh release. As a response to increased ACh levels, AChE activity levels increase to dampen the effect.

Table 1

Participant characteristics

	AD	Non AD
Number of Participant	29	35
Age at Sample	66.8 ±8.6	63.7 ±7.4
Sex	20M,9F	21M,14F
Education	16.5 ±3.2	15.7 ±2.9
MMSE	19.6 ±8.2	23.2 ±7.7
CDR (0,0.5,≥1)	1,8,20	6,12,16 (1missing data)
ApoE4 carrier/non carrier	18/29	8/35
Vascular index	1.3±1.4	1.1±1.1
PIB index	1.6±0.3	1.2±0.3
PIB positive (PIB index>1.2)	27/29	10/35
Interval between plasma sample & PIB (years)	0.6 ± 0.7	0.7 ± 0.7
ChE-I use	20/29 (69%)	12/35 (34%)
Donepezil: Rivastigmine: Galantamine: none	13:3:4:9	8:2:2:23

Table 2

Results of multi linear regression, examining the relationship between PIB index and cholinesterase activity.

	AChE activity			BChE activity		
	Standardized coefficient β	**T**	**Sig.**	**Standardized coefficient β**	**t**	**Sig.**
PIB index	.389	3.784	.000	−.123	−.880	.383
Sex	−.082	−.939	.352	.286	2.211	.031
ApoE4 carrier	−.093	−.991	.326	−.211	−1.524	.134
Age at sample	.073	.828	.411	−.109	−.838	.406
Vascular index	.105	1.196	.237	.049	.377	.708
Donepezil	.606	6.364	.000	-	-	-
Rivastigmine	−.199	−1.994	.051	-	-	-
Galantamine	.071	.783	.437	-	-	-

Table 3

Results of multi linear regression examining the relationship between PIB binding and cholinesterase activity in different ROIs

ROI	AChE activity			BChE activity		
	Standardized coefficient β	T	Sig.	Standardized coefficient β	t	Sig.
Frontal cortex	0.371	3.56	0.001	−0.109	−0.778	0.440
Hippocampus	0.170	1.662	0.102	0.038	0.284	0.778
Lateral temporal cortex	0.378	3.770	<0.001	−0.127	−0.933	0.355
Medial temporal cortex	0.295	2.977	0.004	−0.137	−1.029	0.308
Occipital cortex	0.319	3.323	0.002	−0.195	−1.476	0.146
Parietal cortex	0.412	4.050	<0.001	−0.156	−1.121	0.268
Posterior cingulate cortex	0.387	4.208	<0.001	−0.126	−0.938	0.352
Precuneus	0.382	3.691	0.001	−0.159	−1.144	0.258