PAPER

Degree of inhibition of cortical acetylcholinesterase activity and cognitive effects by donepezil treatment in Alzheimer's disease

N I Bohnen, D I Kaufer, R Hendrickson, L S Ivanco, B J Lopresti, R A Koeppe, C C Meltzer, G Constantine, J G Davis, C A Mathis, S T DeKosky, R Y Moore

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See end of article for authors' affiliations

Correspondence to: Dr Bohnen, University of Pittsburgh, Liliane S Kaufmann Building, Suite 811, 3471 Fifth Avenue, Pittsburgh, PA 15213, USA; nbohnen@pitt.edu

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Objectives: To determine in vivo cortical acetylcholinesterase (AChE) activity and cognitive effects in subjects with mild Alzheimer's disease (AD, n = 14) prior to and after 12 weeks of donepezil therapy.
Methods: Cognitive and *N*-[^{11}C]methyl-piperidin-4-yl propionate ([^{11}C]PMP) AChE positron emission tomography (PET) assessments before and after donepezil therapy.
Results: Analysis of the PET data revealed mean (temporal, parietal, and frontal) cortical donepezil induced AChE inhibition of 19.1% (SD 9.4%) ($t = -7.9$; $p<0.0001$). Enzyme inhibition was most robust in the anterior cingulate cortex (24.2% (6.9%), $t = -14.1$; $p<0.0001$). Donepezil induced cortical inhibition of AChE activity correlated with changes in the Stroop Color Word interference scores ($R^2 = 0.59$, $p<0.01$), but not with primary memory test scores. Analysis of the Stroop test data indicated that subjects with AChE inhibition greater than the median value (>22.2%) had improved scores on the Stroop Color Word Test compared with subjects with less inhibition who had stable to worsening scores ($t = -2.7$; $p<0.05$).
Conclusions: Donepezil induced inhibition of cortical AChE enzyme activity is modest in patients with mild AD. The degree of cortical enzyme inhibition correlates with changes in executive and attentional functions.

Based on the ''cholinergic hypothesis'' of memory, cholinesterase inhibitor (ChE-I) agents (tacrine, donepezil, rivastigmine, galantamine) have been developed to ameliorate cognitive symptoms in Alzheimer's disease (AD).[1–3] However, the effects of these agents on the core cognitive symptoms of AD, particularly short term memory, have been generally modest and variable.[4] The variable efficacy of ChE-I treatment in individual subjects with dementia is not well understood. The recent development of positron emission tomography (PET) technology for measuring cerebral AChE activity in vivo offers the prospect of identifying biological correlates of treatment responsivity to these drugs.[5] AChE activity in the human AD brain has been mapped using PET and radiolabelled acetylcholine analogues, such as *N*-[^{11}C]methyl-piperidin-4-yl propionate ([^{11}C]PMP) and *N*-[^{11}C]methyl-piperidin-4-yl acetate ([^{11}C]MP4A).[6 7] The direct central effect of donepezil hydrochloride (Eisai, Teaneck, NJ), a specific inhibitor of AChE, has been studied in patients with AD using AChE PET imaging. Kuhl and colleagues using [^{11}C]PMP found 27% inhibition of cortical AChE after a minimum of eight weeks of donepezil treatment.[7] [^{11}C]MP4A PET studies found similar or slightly greater cortical AChE inhibition by donepezil in patients with AD (29–39%).[8 9] These studies showed relatively limited brain AChE inhibitory response to therapeutic doses of donepezil when compared with the 70–90% inhibition found in peripheral red blood cells.[8–11] Furthermore, these data showed significant variability in treatment induced enzyme inhibition among subjects with AD.[9 11] Therefore, clinical ChE-I treatment responsiveness may be determined by the degree of cerebral enzyme inhibition.

The primary aim of this study was to compare in vivo cortical AChE activity prior to and after 12 weeks of donepezil therapy in subjects with mild AD. We hypothesised that cortical enzyme inhibitory response to donepezil is modest in this population. We also assessed whether treatment effects on specific cognitive functions were associated with the degree of treatment induced inhibition of cortical AChE activity.

METHODS

Subjects

The study included 14 subjects with mild AD (10 women, four men; mean age 75.1 (SD 5.8) years; Mini-Mental State Examination (MMSE) score 22.6 (SD 4.3)). The subjects met the NINCDS-ADRDA criteria for dementia[12] and none of them was taking anticholinergic medications. The subjects were recruited from the Alzheimer's Disease Research Center at the University of Pittsburgh, Pittsburgh, USA. Each subject underwent a comprehensive neurological and neuropsychological examination. The study was approved by the Institutional Review Board of the University of Pittsburgh.

AChE PET imaging

The [^{11}C]PMP radioligand is an acetylcholine analogue that serves as a selective substrate for AChE hydrolysis.[5] The hydrolysed radioligand becomes trapped as a hydrophilic product locally in the brain following the biodistribution of AChE. AChE has been recognised since 1966 as a reliable marker for brain cholinergic pathways.[13 14] [^{11}C]PMP is a selective substrate for AChE, with a specificity of 97% for AChE determined in mouse brain homogenate studies.[5] As hydrolysis of [^{11}C]PMP radioligand by butyrylcholinesterase

Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; AIR, automated image registration; ChE-I, cholinesterase inhibitor; [^{11}C]PMP, *N*-[^{11}C]methyl-piperidin-4-yl propionate; COWA, Controlled Oral Word Association; CVLT, California Verbal Learning Test; LTM, long term memory; MR, magnetic resonance; PET, positron emission tomography, ROI, region of interest; SPGR, spoiled gradient recall; STM, short term memory; TMT, Trail Making Test

is very limited and donepezil is a selective inhibitor of AChE, our methodology is sensitive to assess cholinergic changes related to AChE but not butyrylcholinesterase activity in AD.[5 11 15 16] [^{11}C]PMP was prepared in high radiochemical purity (>95%) by *N*-[^{11}C]methylation of piperidin-4-yl propionate.[17] The average specific activity was 1910 Ci/μmol (range 340–10 060 Ci/μmol), and less than 7.5 micrograms of mass at the time of injection. Dynamic PET scanning was performed for 80 minutes immediately following a bolus intravenous injection of 555 MBq (15 mCi) of [^{11}C]PMP. Emission data were collected in 21 sequential emission scans (6×30 sec; 4×60 sec; 2×90 sec; 4×300 sec; 5×600 sec) in three dimensional imaging mode using an ECAT HR+ tomograph (CTI PET Systems, Knoxville, TN), which acquires 63 transaxial slices (slice thickness 2.4 mm; in-plane resolution 4.1 mm full-width at half maximum over a 15.2 cm axial field-of-view). The scanner gantry was equipped with a Neuro-Insert (CTI PET Systems, Knoxville, TN) to reduce the contribution of scattered photon events.[18] An individually moulded, thermoplastic mask was made for each subject to minimise head movement and facilitate accurate head positioning. The head was positioned such that the lowest scanning plane (visualised by a system of laser lines within the scanner gantry) was parallel to and 2.0 cm below the canthomeatal line. Prior to [^{11}C]PMP injection, a 10–15 minute transmission scan was acquired using rotating rods of [^{68}Ge/^{68}Ga] for attenuation correction of emission data. PET emission data were also corrected for radioactive decay and scatter and reconstructed using a Hanning filter with a frequency cut-off of 0.5 Nyquist. PET imaging was done prior to and after 12 weeks of donepezil treatment. The time interval between the last dose of donepezil and time of injection of the radioligand for the 12 week PET study ranged from 10 to 16 hours.

Magnetic resonance imaging

A volumetric spoiled gradient recall (SPGR) magnetic resonance (MR) image was obtained for each subject using a Signa 1.5 Tesla scanner (GE Medical Systems, Milwaukee, WI) with a standard head coil. The coronal SPGR sequence (TE = 5; TR = 25; flip angle = 40°; NEX = 1; slice thickness = 1.5 mm; image matrix = 256×192, FOV = 24 cm) was acquired to maximise contrast among grey matter, white matter, and CSF and provide high resolution delineation of cortical and subcortical structures. The MR data were cropped in preparation for alignment with the PET data using AnalyzeAVW software (BIR, Mayo Foundation, Rochester, MN) by setting all non-brain voxels to zero intensity.

Donepezil treatment and cognitive assessments

Donepezil treatment was started 12 weeks before the second PET scan at a dose of 5 mg/day for four weeks, then increased to 10 mg/day. Three visits were conducted during the study: at baseline and during weeks six and 12. Baseline and week 12 testing data were used for analysis for more direct comparison with the PET treatment changes. The cognitive test battery included measures of short and long term memory (California Verbal Learning Test, CVLT STM and LTM), word fluency (Controlled Oral Word Association, COWA), and attention and executive functions (Stroop Color Word interference test and Trail Making Test, TMT B).[19–21] Neuropsychological testing was performed within 24 hours of the 12 week PET scan with the exception of two subjects where the time interval was one week or less.

Data analysis

Prior to coregistration with the cropped MR image, the frames of the first and 12 week repeat dynamic [^{11}C]PMP PET dataset were individually aligned using the automated image registration (AIR) algorithm of Woods *et al* to eliminate interframe registration errors attributable to patient movement.[22] In this procedure, the first seven frames (four minutes) of data were summed and aligned to a centred image of a specified reference frame (frame 15). The resulting single transformation matrix was used to reslice each of the first seven frames because their individual transformation matrices were marred by a poor signal to noise ratio. For all other frames excluding the reference frame, the AIR algorithm was used to register each individual frame, resulting in a separate transformation matrix for each frame, used to reslice only that frame. After registration, the frames were reassembled into a single dynamic dataset where all 21 frames were centred and registered to frame 15. A 5×5 pixel block-smoothing kernel was used in the registration of each frame to reduce high frequency noise and improve performance. The smoothing was used only for coregistration purposes and was not preserved in the output image. The MR image was registered to the PET data using a modified version of AIR that has been validated in our laboratory.[23]

The registered MR and the atlas of Talairach and Tournoux were used to identify regions of interest (ROIs).[24] Cortical ROIs were drawn on the individual subjects' MR images. The frontal ROI included dorsolateral prefrontal association (five slices) and anterior cingulate cortices (seven slices). The parietal ROI included both superior (four slices) and inferior posterior (four slices) lateral parietal association cortices. The posterior cingulate cortex was drawn separately in seven slices. The lateral temporal ROI included the superior (four slices) and inferior (three slices) lateral association cortices. All MR-drawn ROIs were transferred to the PET data for regional sampling of radioactivity. Mean cortical [^{11}C]PMP k3 activity was calculated as a composite score from the frontal, parietal, posterior cingulate, and lateral temporal cortices. Example ROIs are shown in fig 1.

A non-invasive kinetic analysis of the k3 hydrolysis rate (AChE activity) was performed using a direct estimation of k3, without use of an arterial input function, based on the

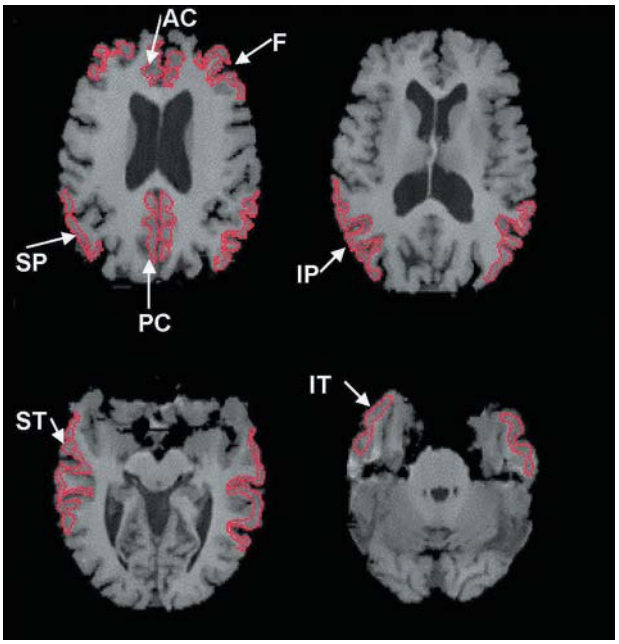

Figure 1 Examples of regions of interest are shown on spoiled gradient recall magnetic resonance images. AC, anterior cingulate; F, dorsolateral prefrontal cortex; IT inferior lateral temporal; IP, inferior lateral parietal; SP, superior lateral parietal; ST, superior lateral temporal.

shape of the tissue time–activity curve alone.[25] The shape analysis has been compared with the more standard compartmental analysis using arterial input functions and non-linear least squares estimation and showed that the non-invasive shape analysis approach gave very similar results to kinetic analysis in the brain cortex.[25] An advantage of the shape analysis approach, which inherently is entirely insensitive to the scale of the data, is that it is nearly unaffected by tissue atrophy.[25] The shape analysis method has also been found to be a sensitive technique for detecting cortical AChE changes in subjects with dementia.[26] Mean values were calculated for both hemispheres.

Paired Student's *t* test was used for comparison of k3 values between scans 1 and 2. Wilcoxon's signed rank test was used for comparison of cognitive test scores at baseline and 12 weeks. The cognitive parameters included CVLT STM and LTM, COWA summed scores for three letters, Stroop Color Word Interference scores at 45 seconds, and TMT B times. Stepwise regression analysis was used to identify cognitive parameters significantly related to cerebral AChE enzyme inhibition and the treatment difference scores between week 12 and baseline of the cognitive tests using the SAS program (SAS Institute Inc., Cary, NC). Absolute differences between baseline and week 12 of the PET and cognitive measures were used. The cognitive parameter with the most significant prediction in the model was then selected for a post hoc analysis to evaluate the nature of the statistical association.

RESULTS

Donepezil induced AChE inhibitions for the cortical regions are shown in table 1. The average cortical (temporal, parietal, and dorsolateral prefrontal) donepezil induced AChE inhibition was 19.1% (SD 9.4%) compared with baseline activity ($t = -7.9$; $p<0.0001$), median value 22.2%. None of the left–right hemispheric differences was significant. A plot of the individual cortical AChE activity levels before and at 12 weeks of donepezil treatment demonstrates an inhibitory response in all subjects (fig 2).

The anterior cingulate area had the most consistent enzyme inhibition (mean 24.2% (6.9%), $t = -14.1$; $p< 0.0001$; table 1). No significant correlation was found between pretreatment cortical AChE activity and treatment induced enzyme inhibition ($R = 0.35$, not significant).

Complete cognitive data were available for 11 subjects (one subject refused repeat testing and data in two subjects were incomplete because of colour blindness). Analysis of cognitive data did not reveal significant group treatment effects for any of the variables (table 2). As we had done direct PET assessment of the biological substrate of the study drug (that is, AChE enzyme inhibition), we examined the cognitive PET data relations to determine whether specific cognitive parameters changed as a function of AChE inhibition during donepezil treatment. For this purpose, we performed a stepwise regression analysis using cortical AChE enzyme inhibition to compare the difference scores between week 12 and baseline for the cognitive tests.

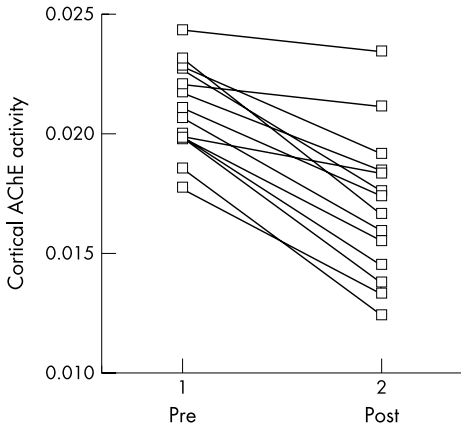

Figure 2 Cortical AChE activity before and at 12 weeks of donepezil treatment in subjects with Alzheimer's disease.

Stepwise multiple regression analysis demonstrated that changes on the Stroop Color Word interference ($R^2 = 0.59$, $p<0.01$) and Trail Making Test B scores ($R^2 = 0.20$, $p<0.05$; total model $R^2 = 0.79$) correlated significantly with donepezil induced inhibition of cortical AChE activity. Changes in PET AChE inhibition did not correlate with scores on other cognitive tests. The individual Stroop Color Word Interference and AChE data are plotted in fig 3 ($r = -0.77$, $p<0.01$).

Analysis of the Stroop test data indicated that subjects with cortical AChE inhibition greater than the median value (>22.2%) had improved scores on the Stroop Color Word Test compared with subjects with less inhibition who had stable to worsening scores (2.2 (4.0) *v* −4.0 (3.7), respectively; $t = -2.7$, $p<0.05$). Stepwise regression analysis limited to the anterior cingulate cortex demonstrated similar cognitive associations when compared with the mean cortical AChE activity: Stroop Color Word interference ($R^2 = 0.55$, $p<0.01$) and Trail Making Test B scores ($R^2 = 0.22$, $p<0.05$; total model $R^2 = 0.75$).

DISCUSSION

We found a modest degree of cortical AChE inhibition to donepezil in subjects with mild AD. Enzyme inhibition was most robust in the anterior cingulate cortex followed by the dorsolateral prefrontal and posterior cingulate cortices. Our findings are in agreement with the study of Kuhl and colleagues who noted relatively limited brain AChE inhibitory response to therapeutic doses of donepezil when compared with peripheral red blood cell enzyme inhibition.[10 11] This could reflect peripheral mechanisms, such as absorption or metabolism, but also raises the possibility of limited or variable blood–brain barrier passage or differences in central

Table 1 Regional cortical [^{11}C]PMP k3 hydrolysis rates (mean (SD)) before and after 12 weeks of donepezil therapy in subjects with Alzheimer's disease

Rate of [^{11}C]PMP k3 hydrolysis (per minute)	Pretreatment	Post-treatment	% Inhibition	Paired *t* test
Mean cortical	0.0210 (0.0019)	0.0170 (0.0031)	19.1 (9.4)	$t = -7.9$; $p<0.0001$
Dorsolateral prefrontal	0.0221 (0.0025)	0.0172 (0.0025)	22.2 (8.3)	$t = -10.8$; $p<0.0001$
Parietal	0.0203 (0.0019)	0.0170 (0.0031)	16.3 (9.9)	$t = -6.6$; $p<0.0001$
Lateral temporal	0.0199 (0.0022)	0.0166 (0.0035)	16.6 (14.1)	$t = -4.4$; $p<0.001$
Anterior cingulate	0.0231 (0.0023)	0.0175 (0.0028)	24.2 (6.9)	$t = -14.1$; $p<0.0001$
Posterior cingulate	0.0218 (0.0024)	0.0170 (0.0031)	22.6 (9.7)	$t = -8.7$; $p<0.0001$

Paired *t* test scores are presented with levels of significance.

Table 2 Cognitive test scores (mean (SD)) prior to and during 12 weeks of donepezil therapy

	Baseline	12 weeks	% Change	Wilcoxon's signed rank test
MMSE	22.6 (4.3)	23.5 (3.4)	4.0 (16.4)	S=11.5, NS
CVLT-STM	2.0 (2.4)	2.5 (2.3)	25.0 (108.1)	S=5, NS
CVLT-LTM	2.1 (2.5)	2.9 (3.7)	38.1 (124.5)	S=8.5, NS
TMT B (seconds)	215.9 (82.9)	227.5 (80.00)	5.4 (32.6)	S=11.5, NS
Stroop Interference	15.9 (6.0)	15.5 (5.7)	−2.5 (27.1)	S=−8, NS
COWA	28.8 (13.4)	30.3 (10.4)	5.2 (42.2)	S=5, NS

Wilcoxon's signed rank test S values with significance levels are presented.
Attention and executive functions (Stroop Color Word interference test and Trail Making Test, TMT B).
Word fluency (Controlled Oral Word Association, COWA).
CVLT, California Verbal Learning Test; MMSE, Mini-Mental State Examination; S/LTM short/long term memory.

metabolism of the drug.[10 11] Steady-state pharmacokinetics of donepezil are reached within 14–22 days after repeated administration of 5 mg or 10 mg daily.[16] Therefore, is plausible that cerebral enzyme inhibition under steady-state pharmacokinetic conditions may be significantly different from acute single dose pharmacological exposure.[27] Our findings are also in agreement with Kaasinen *et al* who noted relatively more enzyme inhibition by donepezil in the frontal compared to the parietal and temporal cortical regions.[9] This may reflect regional variability in cholinergic innervation of the human cortex.[28]

We found that donepezil induced inhibition of cortical AChE activity in subjects with AD correlated with changes in executive and attentional but not primary memory functions. Analysis of drug trials using the ChE-I tacrine have shown that cognitive parameters of attention and executive functions improved more after treatment than did mnemonic functions.[29 30] Overall, most clinical trials of ChE-I drugs have shown improved scores on global measures of cognitive abilities, such as the MMSE, the cognitive subscale of the Alzheimer Disease Assessment Scale, and a global scale such as Clinician Interview-Based Impression scale.[3] These broad effects on cognition suggests that cholinergic agents may have a primary influence on executive or attentional systems with a secondary general modulatory effect on memory, language, and visuospatial skills.[3 31] Conversely, anticholinergic drugs have disproportionately adverse effects on executive processes, attention, and working memory.[32] For example, Dubois *et al* reported that the use of anticholinergic medications in patients with Parkinson's disease led to worsening executive and attentional functions as assessed by the Wisconsin card sorting task and digit span test.[33 34] Global effects of ChE-I drugs may, therefore, relate in part to their influence on executive and attentional functions.

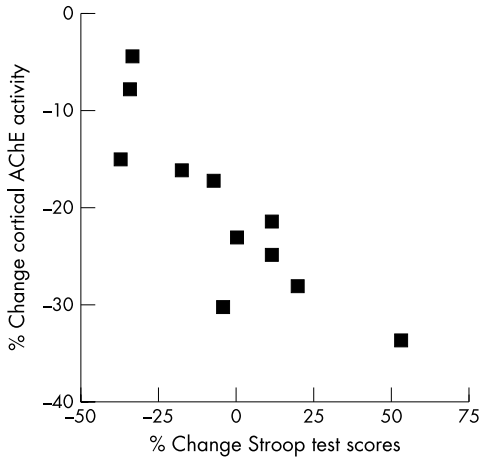

Figure 3 Per cent inhibition of cortical AChE activity during donepezil therapy plotted against per cent change in treatment performance on the Stroop Color Word Test. Positive per cent change on the Stroop test represents improved performance.

On analysis of the Stroop test data we found improved colour word interference scores in the subset of subjects with higher enzyme inhibition, whereas subjects with lower inhibition had stable to worsening test scores. These findings provide evidence for a threshold effect of AChE inhibition needed for the detection of therapeutic efficacy of donepezil on specific cognitive tests. It remains to be studied whether a subset of subjects without a beneficial response to donepezil may have a drug induced decrease rather than an increase in cerebral blood flow.[35]

The present study had some limitations: the open label treatment design, relatively short duration of the study, and the small sample size. However, direct PET assessment of the biological substrate of the study drug (that is, AChE enzyme inhibition) provides a unique way to evaluate individual treatment responsivity.

In conclusion, this study demonstrates that donepezil induced inhibition of cerebral AChE enzyme activity is modest in subjects with early AD. Further research is needed to investigate possible threshold effects of cerebral AChE inhibition by donepezil in relation to its clinical efficacy, reasons for the modest degree of the inhibition, explore dose–response relation at higher dose levels, and direct comparison between peripheral red blood cell and cerebral AChE inhibition.

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Authors' affiliations

N I Bohnen, D I Kaufer, R Hendrickson, L S Ivanco, S T DeKosky, R Y Moore, Department of Neurology, University of Pittsburgh Medical School, Pittsburgh, PA, USA
N I Bohnen, B J Lopresti, C C Meltzer, J G Davis, C A Mathis, Department of Radiology, University of Pittsburgh Medical School, Pittsburgh, PA, USA
N I Bohnen, VA Pittsburgh Healthcare system, Pittsburgh, PA, USA
R A Koeppe, Department of Radiology, The University of Michigan, Ann Arbor, MI, USA
G Constantine, Department of Mathematics and Statistics, University of Pittsburgh, Pittsburgh, PA, USA

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