Brain Perfusion Follow-Up in Alzheimer's Patients During Treatment with Acetylcholinesterase Inhibitors

Flavio Nobili, MD[1]; Malick Koulibaly, PhD[2]; Paolo Vitali, MD[1]; Octave Migneco, MD[2]; Giuliano Mariani, MD[3]; Klaus Ebmeier, MD[4]; Alberto Pupi, MD[5]; Philippe H. Robert, MD, PhD[6]; Guido Rodriguez, MD[1]; and Jacques Darcourt, MD, PhD[2]

[1]Clinical Neurophysiology, Department of Internal Medicine, University of Genoa, Genoa, Italy; [2]Nuclear Medicine Department, Laboratory of Biophysics, Centre Antoine Lacassagne, University of Nice, Sophia Antipolis, France; [3]Nuclear Medicine, Department of Internal Medicine, University of Genoa, Genoa, Italy; [4]Department of Psychiatry, University of Edinburgh, Edinburgh, United Kingdom; [5]Department of Physiopathology, University of Florence, Florence, Italy; and [6]Memory Center, Federation of Clinical Neuroscience, CHU-University of Nice, Sophia Antipolis, France

Transient cognitive and behavioral stabilization of patients with Alzheimer's disease (AD) is the main goal of long-term acetylcholinesterase inhibitor (AChEI) therapy, but response to treatment is variable and, indeed, only some of the patients are stabilized. This is usually assessed by means of clinical and neuropsychologic scales, whereas functional neuroimaging could allow objective evaluation of the topographic correlates of the effect of therapy on brain functioning. The aim of this study was to evaluate brain perfusion changes by SPECT in AD patients during chronic AChEI therapy in relation to their cognitive evolution. **Methods:** Forty-seven consecutive outpatients with mild-to-moderate probable AD (as defined by the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association and the *Diagnostic and Statistical Manual of Mental Disorders* [4th edition criteria] and a score of ≥15 on the Mini-Mental State Examination [MMSE]) were enrolled in 2 centers over a 1-y period and underwent SPECT with ^{99m}Tc-hexamethylpropyleneamine oxime at the time of enrollment (t_0). All of them started AChEI therapy. Nine patients were lost at follow-up, and drugs were withdrawn from 3 patients. Of the remaining 35 patients, who received regular AChEI therapy (donepezil, 5 or 10 mg/d; rivastigmine, 6 or 9 mg/d) throughout the observation period, only the 31 patients receiving donepezil were considered to avoid the possible confounding effect of different drugs. The 31 patients completed the study and a second SPECT examination was performed 15.0 ± 3.0 mo later (t_1). They were divided into stabilized (17 patients) and nonstabilized (14 patients) subgroups on the basis of the minimum expected annual rate of decline of the MMSE score, derived from a meta-analysis of the literature. SPECT data were analyzed by means of statistical parametric mapping. **Results:** At baseline, the stabilized and nonstabilized patients were comparable for age, sex distribution, education, MMSE scores, memory impairment (selective reminding test [SRT]), apolipoprotein E genotype, AChEI dose regimen, and SPECT findings. The SRT scores decreased significantly ($P < 0.01$) in the nonstabilized subgroup but not in the stabilized subgroup. No significant difference was found between the baseline and repeated SPECT data in the stabilized subgroup. In contrast, in the nonstabilized subgroup a significant perfusion reduction was found in the frontal, temporal, and parietal superficial cortex and in the occipital precuneus in the right hemisphere and in the frontal and mesial temporal cortex in the left hemisphere. On repeated SPECT, regional cerebral blood flow was significantly lower in a left frontal region in the nonstabilized group than in the stabilized group. **Conclusion:** The regional cerebral blood flow decreases in several cortical regions in AD patients with cognitive deterioration despite long-term AChEI therapy, similar to that observed in untreated patients, whereas it remains stable in AD patients with stabilized cognitive performance during therapy.

Key Words: SPECT; regional cerebral blood flow; Alzheimer's disease; acetylcholinesterase inhibitors; donepezil

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Alzheimer's disease (AD) is characterized clinically by cognitive and behavioral disorders that interfere significantly with activities of daily living. These disorders result from structural and functional alterations in several brain areas, mainly in the association cortex. Functional neuroimaging methods (such as PET and SPECT) are widely used in the diagnosis of AD in addition to conventional structural neuroimaging. Both methods yield cerebral functional deficit in AD, typically in the temporoparietal area (*1*,*2*), the posterior cingulate (*3*,*4*) and precunei (*4*), and the hippocampal region (*5*), although their clinical role in increasing diagnostic accuracy is still a matter of debate (*6*,*7*).

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For correspondence contact: Flavio Nobili, MD, Clinical Neurophysiology, Department of Internal Medicine, University of Genoa, Viale Benedetto XV, 6, I-16132 Genoa, Italy.

E-mail: fnobili@smartino.ge.it

In AD, the cholinergic system is one of the most consistently and profoundly affected central neurotransmission systems. The number of cholinergic neurons originating in the nucleus basalis of Meynert and projecting their axons to the cerebral cortex is markedly decreased (*8*). As a result, the concentrations of acetylcholine (ACh) and choline acetyltransferase are strongly reduced in the cerebral neocortex and hippocampus (*9*). This putative pathogenic mechanism led to the introduction of acetylcholinesterase inhibitors (AChEIs) for the treatment of AD, such as tacrine and, more recently, donepezil, rivastigmine, metrifonate, and galantamine, with the aim of improving synaptic cholinergic transmission in the brain.

However, the clinical response to AChEI therapy is variable (*10*) and has generally been assessed in terms of stabilization or slowed deterioration of cognitive function and daily activities over a relatively short period (in comparison to the overall disease course). These studies have been based on clinical and neuropsychologic scales (*11*). Functional neuroimaging should be able to identify functional changes in the brain more directly. Indeed, if the clinical response to AChEIs in AD is mediated by brain synaptic cholinergic activation, this will be reflected by better-preserved neuronal activity along cholinergic pathways and, thus, by higher metabolic and blood flow requirements (*12*). Recently, better regional cerebral blood flow (rCBF) preservation in some brain regions has been reported in mild-to-moderate AD patients receiving donepezil (5 mg/d) for 1 y, compared with an AD group receiving a placebo (*13*), but no data are available as yet on patients with different disease evolution while on therapy with AChEIs.

We used SPECT to evaluate brain perfusion changes in patients with mild-to-moderate AD during long-term AChEI administration according to their cognitive changes.

MATERIALS AND METHODS

Patients

As part of the 3-y European Union cooperative study entitled SPECT in Dementia, which started in March 1998 and involves 4 European countries (France, Germany, United Kingdom, Italy), 2 centers in Nice (France) and Genoa (Italy) developed a common subproject aiming to reassess AD patients by means of brain SPECT, 12–18 mo (t_1) after the baseline examination (t_0).

Forty-seven consecutive outpatients with mild-to-moderate probable AD were enrolled by the 2 centers during the first year of the study. All patients or their relatives were informed of the aim of the study and gave their consent. AD was diagnosed according to current standards (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (*14*) and the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition criteria [DSM-IV]) (*15*). The Hachinski ischemic score was <4 in all patients. A questionnaire was used to rule out frontotemporal dementia (*16*), with all patients with scores of >0 being excluded. Other exclusion criteria were (a) current DSM-IV psychiatric disorders other than dementia; (b) electroconvulsive therapy within the last 6 mo; (c) evidence of organic brain disorders such as Parkinson's disease, epilepsy, and so forth; (d) any history of significant head injury with prolonged loss of consciousness or skull fracture; (e) major medical illnesses potentially interfering with the study evaluation, including malignancies, poorly controlled diabetes mellitus, renal or hepatic failure, serious heart disease, and severe inflammatory disease; (f) recent changes in medication likely to have an effect on rCBF; and (g) history of drug or alcohol dependence within the last 10 y.

Mild-to-moderate AD was defined by a Mini-Mental State Examination (MMSE) (*17*) score of ≥ 15 at baseline. All patients also underwent the Selective Reminding Test (SRT) (version of Grober et al. (*18*) in Nice; version of Masur et al. (*19*) in Genoa); the score was obtained by summing the number of words recalled for each item. The SRT score was then normalized to age- and sex-matched healthy individuals in each laboratory by calculating the normal standard deviate *z*.

The 47 patients comprised 31 women and 16 men (age, 53–83 y; mean age, 73.1 $\pm$ 6.9 y). The main clinical and neuropsychologic baseline characteristics are summarized in Table 1. Nine patients were lost to follow-up, either because they failed to attend the second clinical examination or because they refused the second SPECT examination. Of the remaining 38 patients who underwent repeated SPECT and the same clinical and neuropsychologic assessment as at baseline, 3 were not taken into consideration in this study because they were not on AChEI therapy (drugs were withdrawn because of side effects or other social reasons). Thus, 35 patients regularly received AChEIs (donepezil, $n = 31$; and rivastigmine, $n = 4$). To avoid the possible confounding effect of 2 different drugs, only the patients receiving donepezil were analyzed further. Therefore, the final study group consisted of 31 patients (20 in Genoa and 11 in Nice), whose main characteristics are also summarized in Table 1.

The patients from Nice had a milder degree of dementia than the patients from Genoa at the time of enrollment. In fact, they had a higher ($P < 0.05$) MMSE score (22.5 $\pm$ 4.3) than that of the Genoa patients (19.9 $\pm$ 3.3) and a shorter ($P < 0.05$) duration of disease (26.2 $\pm$ 10.5 mo) than that of the Genoa patients (41.0 $\pm$ 22.8 mo).

All 31 patients regularly received oral donepezil (5 mg/d, 17 patients; 10 mg/d, 14 patients) throughout the study. The patients' physicians chose the drug and the dose regimen on the basis of their clinical experience. All but 2 of these 31 patients were not taking AChEIs at the baseline examination.

The 31 patients were divided into 2 subgroups on the basis of the MMSE score change between t_0 and t_1. A meta-analysis of the literature, including 3,492 patients enrolled in 37 studies published between 1988 and 1997 (before widespread use of AChEIs), showed a mean annual MMSE score decline of 3.3 points, with a 95% confidence interval of 0.4 (*20*). Therefore, it was assumed that most untreated AD patients would lose at least 2.9 points on the MMSE per year. The expected minimum overall MMSE score change in each patient was thus computed as (2.9/12) $\times$ the interval in months between t_0 and t_1. If the MMSE score fell by more than this value, the patient was considered to be nonstabilized (nS). In contrast, if the MMSE score fell by less than this value, or if it remained stable or increased, the patient was considered to be stabilized (S). On the basis of these criteria, the S subgroup had 17 patients and the nS subgroup had 14 patients. The main clinical features of these 2 subgroups are given in Table 1.

TABLE 1
General Characteristics of Patients with Mild-to-Moderate AD

Characteristic	Initial group	Study group	nS subgroup	S subgroup
Patients (no.)	47	31	14	17
Sex (F/M)	31/16	19/12	10/4	9/8
Age* (y)	73.1 ± 6.9	73.5 ± 6.1	71.3 ± 6.6	73.8 ± 5.9
Years of education*	8.8 ± 4.5	8.1 ± 4.8	7.5 ± 4.7	8.6 ± 5.0
Duration of disease* (mo)	33.4 ± 18.9	35.7 ± 20.4	33.5 ± 20.9	37.6 ± 20.5
Apo E† (%)	53	51.6	50	58.8
t_0 MMSE score*	21.6 ± 4.4	20.9 ± 3.8	19.8 ± 3.3	21.8 ± 4.1
t_1 MMSE score*		18.4 ± 5.4	14.9 ± 4.3‡	21.2 ± 4.6§
t_0 SRT score* (*z* values)	−2.9 ± 1.8	−3.2 ± 1.4	−3.3 ± 1.0	−3.0 ± 1.7
t_1 SRT score* (*z* values)		−3.6 ± 1.6	−4.1 ± 1.3‡	−3.2 ± 1.7¶
Interval* (mo) between t_0 and t_1		15.0 ± 3.0	13.9 ± 2.3	15.8 ± 3.4

*Mean ± SD.
†At least 1 ϵ4 allele.
‡nS at t_1 vs. nS at t_0: $P < 0.01$ (paired *t* test).
§nS vs. S: $P < 0.01$ (unpaired *t* test).
¶nS vs. S: $P < 0.05$ (unpaired *t* test).
nS = patients clinically nonstabilized by AChEI; S = patients clinically stabilized by AChEI.

Four patients in the S subgroup and 2 patients in the nS subgroup were on treatment with low-dose neuroleptics, benzodiazepines, or meprobamate, and no change in therapy was undertaken during the follow-up period. Mild-to-moderate arterial hypertension was present in 5 patients in the S subgroup and in 3 patients in the nS subgroup; they were treated with calcium antagonists or angiotensin-converting enzyme inhibitors with good control of the blood pressure values.

SPECT Studies

Acquisition and Reconstruction Protocols. The protocol was optimized to make resulting images as comparable as possible. In the Nice center, 120 projections of 55 s were gathered using a 3-head gamma camera (Prism 3000 XP; Picker International, Cleveland, OH) equipped with low-energy, ultrahigh-resolution fanbeam collimators (focus, 50 cm). The radius of rotation was 13.3 cm. During acquisition, the patient's head was immobilized with a head holder. Reconstruction was performed by filtered backprojection (FBP) with 3-dimensional postfiltering (Butterworth filter: order, 6; cutoff frequency, 0.33) on 128 × 128 pixel matrices. The voxel size was 2.2 mm in each direction (*x, y, z*). This yielded a spatial resolution (full width at half maximum [FWHM]) of 8 mm on the reconstructed cuts. Chang's first-order attenuation correction method (*21*) was applied to the data; the attenuation coefficient was 0.07/cm.

In the Genoa center, 120 projections of 15 s were acquired with a brain-dedicated camera (CERASPECT; Digital Scintigraphics, Waltham, MA). The gamma camera was equipped with a stationary NaI(Tl) annular crystal and a cylindric, low-energy, high-resolution lead collimator, which is the only moving part. The radius of rotation was 15 cm. Sixty-four axial slices were reconstructed on 128 × 128 pixel matrices using FBP with 2-dimensional prefiltering (Butterworth filter: order, 10; cutoff frequency, 0.6). The voxel size was 1.7 mm in each direction (*x, y, z*). This yielded a spatial resolution (FWHM) of 8 mm on the reconstructed cuts (i.e., comparable with that achieved with the Prism 3000 XP). Chang's first-order attenuation correction method (*21*) was applied, with an attenuation coefficient of 0.11/cm.

Phantom Studies. Before the study began, data activity from the same JB003 human brain phantom were acquired in the 4 centers participating in the study (i.e., Cologne, Genoa, Nice, and Edinburgh). The phantom used was a high-resolution, 3-dimensional anthropomorphic JB003 phantom (Nuclemed N.V./S.A., Roeselare, Belgium). The phantom was filled with approximately 50 MBq ^{99m}Tc; approximately 5 million counts were acquired during the scanning. For the purpose of this study, phantom scans acquired in Genoa and in Nice were compared (using statistical parametric mapping [SPM99; Wellcome Department of Cognitive Neurology, University College London, London, U.K.]) with the images collected from a group of 15 healthy control subjects (6 men, 9 women; age, 65–86 y; mean age, 74 ± 5.8 y) obtained from the Society of Nuclear Medicine database (http://www.snm.org) and acquired with the same gamma camera as that used in Nice.

Phantom images were reconstructed by FBP and were then corrected for attenuation using the same protocol as for patient scans. Images of the control group were processed in the same way as in Nice. Datasets were spatially normalized and then smoothed with an isotropic gaussian kernel of FWHM 12 mm. For global intensity normalization, a proportional scaling was used. The statistical analysis option of the SPM99 software "compare populations: 1 scan/subject" was used, where group 1 had 1 image (the phantom) and group 2 had 15 control images.

The SPM{*z*} analyses (corrected $P = 0.001$ at cluster level) of the phantom acquired in Genoa and in Nice, respectively, versus the control group showed very similar results for the topography and the extension of differences.

Patient Injection and Acquisition. The injection protocol was identical at the 2 centers. SPECT acquisitions were performed between 30 and 90 min after intravenous injection of 740–925 MBq freshly prepared ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO) (Ceretec; Amersham Medical, Ltd., Amersham, U.K.).

To minimize sensory input, the injection was performed through an intravenous line in a quiet, dimly lit room, with the patient reclining on a chair, eyes closed and ears unplugged. For each examination, 10–12 million counts were acquired in Nice and 7–9 million counts were acquired in Genoa. Each patient's baseline and repeated SPECT examinations were done with the same camera, and each patient was his or her own control.

Statistical Analysis

The 2 subgroups were compared for age, education, disease duration, t_0 and t_1 MMSE score, t_0 and t_1 SRT *z* score, and the interval between the baseline and repeated SPECT examinations using an unpaired *t* test. Moreover, the 2 subgroups were also compared for sex, apolipoprotein E (Apo E) ϵ4 allele, and AChEI dosage distributions using Fisher's exact test. Changes in the MMSE score and the SRT *z* score values between t_0 and t_1 within each subgroup were tested for significance by a paired *t* test.

SPECT data were analyzed using SPM99 (*22*). In a preprocessing step, datasets were spatially normalized and smoothed with an isotropic gaussian kernel of FWHM 12 mm. For global intensity normalization, a proportional scaling was used. SPM{*t*}, the statistical parametric maps of the *t* statistic, were then calculated and thresholded using an uncorrected $P = 0.001$. Because of the lack of an anatomic a priori hypothesis, the significance of identified regions was assessed using probability values corrected for multiple comparisons.

SPM99 coregisters the individual SPECT to the 152-brain average of the Montreal Neurological Institute (http://www.bic.mni.mcgill.ca). Because this template does not completely match the Talairach brain, it is necessary to correct the SPM{*t*} coordinates. This was achieved using the subroutine implemented by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging) that gives the correspondence between SPM and the Talairach and Tournoux atlas coordinates (*23*).

In a preliminary analysis, the 20 Genoa patients were compared with the 11 Nice patients at t_0 because Nice patients had been found to have a milder severity of disease. In fact, an area of higher rCBF in the right temporoparietal superficial cortex was found in the Nice patients in comparison with the Genoa patients.

The S and the nS subgroups at t_0 and t_1 were analyzed by means of a factorial 2×2 design, which was performed with the "Multigroup: conditions and covariates" option of the SPM99 software. This kind of analysis takes into account the 2 factors (i.e., the group [S and nS] and the condition [t_0 and t_1]) and their interactions. Moreover, the dosage of donepezil (5 or 10 mg/d) was considered as a possible confounding effect.

RESULTS

Table 1 summarizes the clinical and demographic characteristics of the original sample of 47 patients, of the final study group, and of the S and nS subgroups.

The S and nS subgroups did not differ in terms of their sex ratio (although there tended to be a larger proportion of women in the nS subgroup), age, education, disease duration, the Apo E ϵ4 genotype prevalence, the basal MMSE score, the basal SRT *z* score, the interval between the baseline and repeated SPECT examinations, and the AChEI dosage. The MMSE score decline in the nS subgroup was merely the consequence of selection criteria of the 2 subgroups. However, in keeping with such criteria, the SRT score as well fell significantly ($P < 0.01$) only in the nS patients.

The factorial 2×2 design showed a significant main effect of the condition (i.e., t_0 and t_1) on the 31 patients as a whole. Four large clusters of significant (corrected $P < 0.001$) rCBF reduction were observed, involving the frontal, temporal, and parietal superficial cortex and the occipital precuneus in the right hemisphere and the frontal and the mesial temporal cortex in the left hemisphere. Statistical significance (corrected $P < 0.05$) was found for all 4 clusters at the voxel level as well. The simple main effect of the condition was absent in the S subgroup, whereas it was highly significant in several clusters in the nS subgroup, with the maximum difference in the right precuneus, right

TABLE 2
Numeric Results of SPM Analysis (Height Threshold: $P = 0.001$)

	Cluster level				Voxel level						
Comparison	Cluster extent	Corrected *P*		Cortical region	*z* score of maximum	Talairach coordinate (*x*, *y*, *z*)			Corrected *P*	Cortical region	BA
$(t_0 - t_1)$nS	8,560	<0.001	R	F, T, P	5.17	63	−53	23	0.004	Supramarginal gyrus	40
	1,405	<0.001	R	P–O, post. cing.	6.36	17	−63	22	<0.001	Precuneus, O lobe	31
	887	<0.001	L	F	4.23	−32	4	33	NS		
	503	0.002	L	T	4.82	−26	2	−32	0.02	Uncus	36
	435	0.005	R	C	4.65	53	−58	−24	0.03	Tuber	
	269	0.03	L	F	4.44	−28	29	−10	NS		
	261	0.03	L	thalamus	4.37	−10	−21	8	NS		
$(S - nS)t_1$	495	=0.02	L	F	3.92	−28	44	−11	NS		

BA = Brodmann's area; F = frontal; T = temporal; P = parietal; O = occipital; post. cing. = posterior cingulate gyrus; NS = not significant; C = cerebellar.

For area of maximum difference, cortical region and BA are reported only if significant at voxel level.

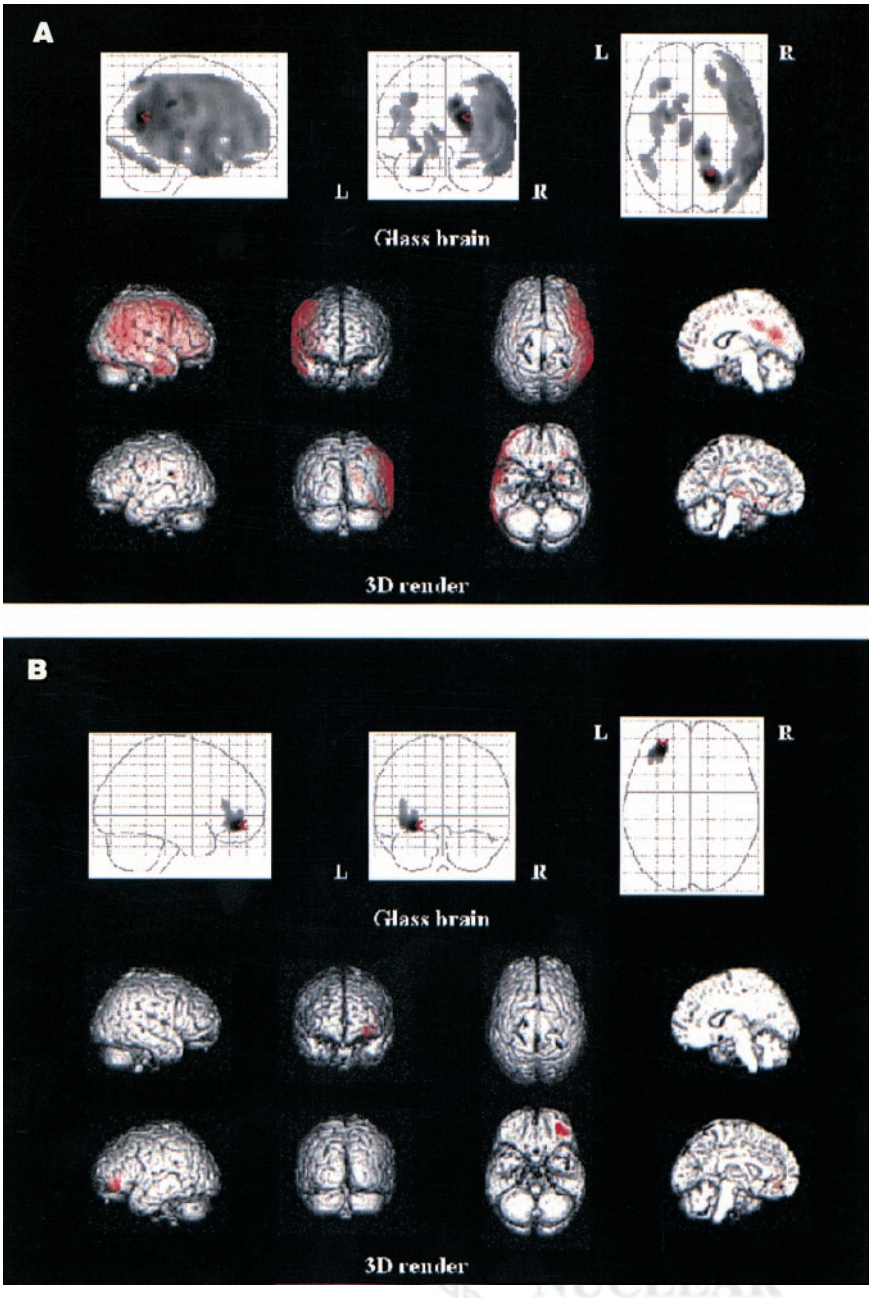

FIGURE 1. (A) *z* map of decreased rCBF on repeated SPECT examination (t_1) compared with baseline examination (t_0) in AD nS subgroup. Significant areas (shown as glass brain in upper part and colored areas superimposed on standard 3-dimensional anatomic template [3D render] in lower part) are found at t_1 in frontal, temporal, and parietal superficial cortex in right hemisphere and in frontal and mesial temporal cortex in left hemisphere. Left thalamus and right cerebellar lobe are involved to some extent. Maximum difference is found in right precuneus at level of occipital lobe (BA 31) ($z = 6.36$; corrected $P < 0.001$ at cluster level; peak Talairach (*23*) coordinates: 17, −63, 22). (B) *z* map of decreased rCBF in nS subgroup compared with S subgroup at t_1. Significant area is found in left frontal cortex (maximum difference in middle frontal gyrus (BA 11) ($z = 3.92$; corrected $P = 0.02$ at cluster level; peak Talairach (*23*) coordinates: −28, 44, −11). Other details as in A.

supramarginal gyrus, left uncus, and right cerebellar tuber (Table 2; Fig. 1A). Thus, several areas of significant rCBF reduction were found in the nS subgroup, whereas no significant rCBF change was present between t_0 and t_1 in the S subgroup.

No significant main effect of the group (i.e., S and nS subgroups) was found in all patients as a whole. The simple main effect of the group was absent at t_0 but was significant at t_1 because rCBF was lower in the nS subgroup than in the S subgroup in a left frontal region (Table 2; Fig. 1B). Finally, a significant ($P < 0.01$ at cluster level) effect of the interaction between the condition (i.e., t_0 and t_1) and the group (i.e., S and nS subgroups) was found in the temporal pole of the right hemisphere.

DISCUSSION

The SPECT brain perfusion pattern was unchanged in AD patients whose cognitive performance was stabilized during long-term AChEI therapy, whereas perfusion reduction was found in several brain regions of both hemispheres in patients whose disease worsened despite AChEI therapy.

Before discussing the results, some sources of inhomogeneity among the selected patients should be considered. First, because the study began in 1998, when donepezil was recommended at 5 mg/d (*10*), some of the patients were still receiving this dosage of donepezil several months after starting therapy, whereas the others were shifted to 10 mg/d. However, donepezil dosage was comparable between sub-

groups and, moreover, the possible confounding effect of different dosage was considered in the statistical analysis. Second, the Nice patients were somewhat less severely demented than the Genoa patients, and this resulted in a higher rCBF in a right parietotemporal region at basal examination. However, the JB003 phantom acquired in the 2 centers gave very similar results between the 2 gamma cameras, and thus it is likely that the rCBF difference between the patients enrolled by the 2 centers was the consequence of the milder severity of dementia of the Nice patients. Finally, although 2 different forms of SRT were applied, raw scores were normalized on the normal reference population of each center by the computation of normal standard deviate *z*, thus reducing the likelihood of introducing a possible bias between the centers.

The 2 subgroups were created, after adjustment for the length of follow-up, on the basis of the expected minimum annual rate of decline of the MMSE score, as estimated by a meta-analysis of studies reporting the natural course of AD (*20*). Other authors have used a similar method, based on the Neuropsychiatric Inventory, to estimate behavioral changes (*24*). Our results appear to support this approach because significant differences between t_0 and t_1 values for SRT scores and perfusion values were observed between the stabilized and nonstabilized patients. Other analytic approaches could have been applied to the issue of rCBF and cognitive changes induced by AChEIs. For example, a parametric design in which the quantified cognitive decline is correlated with the perfusion changes over time would be a suitable one. This kind of analysis would produce an SPM map of correlation between rCBF and MMSE or SRT score changes, thus providing information on brain areas in which rCBF is more suitable to decrease together with cognitive deterioration and vice versa. However, such an approach would not provide a direct answer on whether AD patients with a good cognitive response to AChEIs also have a preserved rCBF over the whole brain (and vice versa), which was the specific objective of this investigation.

A main effect of the condition (i.e., t_0 and t_1) was found, which was accounted for by rCBF decrease in several brain areas in the nS subgroup, whereas no significant effect of condition was observed in the S subgroup, thus meaning that rCBF remained substantially stable between t_0 and t_1 in the S subgroup. The cerebral areas of rCBF reduction with the course of time in nonstabilized patients are already well known to be involved with the worsening of the disease. The regions of the precuneus and of the posterior cingulate gyrus of the right hemisphere were the most affected, thus confirming that these areas are typically involved in mild AD. The right hemisphere was more involved than the left one. In fact, an asymmetric brain involvement has been reported frequently in AD, and either the left or the right hemisphere has alternatively been reported to be the most affected in different studies, although the exact pathophysiologic meaning of such a side-related difference is unclear as yet (*25*). Note that the right cerebellar lobe showed rCBF reduction as well. Such a precise localization should be taken with caution, given the approximation introduced by the SPM normalization procedure. However, this result would be consistent with recent pathologic studies highlighting the previously neglected involvement of infratentorial structures in AD (*26*).

The significant effect of the condition (i.e., t_0–t_1) was found after an average time of 15 mo of AChEI therapy. Our findings are in keeping with several recent randomized, placebo-controlled studies that have consistently shown that the effects of AChEIs on cognition and behavior are still detectable after 1 y of therapy (*27*,*28*), and an even longer effect has been supposed (*29*). It is likely that the rCBF preservation in stabilized patients compared with nonstabilized ones is related at least in part to the effects of AChEI therapy. In fact, increased synaptic ACh availability may improve neurotransmission in cholinergic pathways and, in turn, prevent the AD-associated decline in brain metabolism and blood flow. It has been speculated that donepezil potentiates the formation of new neural connections (*13*). Giacobini (*30*) has suggested that the long-lasting effect of AChEIs on cognitive deterioration in AD may indicate a mechanism of action that would go beyond a merely symptomatic effect. According to this hypothesis, AChEIs might partially reverse the increased deposition of cortical amyloid-precursor protein that follows the lesions of cholinergic nuclei. Serial SPECT studies in the earlier therapeutic stage will lead to a gain of more information on a different rCBF pattern evolution while the patient is on therapy with AChEIs. In fact, an initial response to therapy—or even a second response to increased doses of AchEIs—may be present even in nonstabilized patients.

A simple main effect of the group was observed at t_1 but not at t_0, thus meaning that the 2 subgroups were similar at t_0 but they differed at t_1. The lack of a significant baseline rCBF difference between the 2 subgroups does not permit any pretreatment rCBF pattern characterization. A lower orbito- and dorsolateral frontal cortex rCBF was reported at baseline in patients with a good behavioral response to AChEIs (*24*). However, that study followed the patients for only 8 wk compared with 15 mo of this research. Moreover, the 2 studies cannot be compared directly because our study focuses on cognitive aspects, whereas the other study focused mainly on behavioral changes, which are now known to be influenced positively by AChEI therapy.

A significantly lower rCBF was found in the left lateral frontal cortex at t_1 in nonstabilized patients in comparison with stabilized patients. Because rCBF did not decrease significantly in this region in nonstabilized patients, one should assume this results mainly from a better preservation of rCBF in these regions between t_0 and t_1 in stabilized patients. The most significant relative perfusion increase on SPECT was found in the right frontal lobe in AD patients receiving donepezil for a mean of 35 wk (*31*). Moreover, rCBF preservation has been seen on SPECT in several brain

areas, including the prefrontal cortex, between AChEI-treated AD patients and AD patients receiving a placebo (*13*). Finally, a better improvement in the frontal lobes was shown by quantitative electroencephalography, even after >1 y of tacrine administration, together with an improvement of the trail-making test, a sign of frontal activation (*32*). In keeping with these latter findings, it could be speculated that the effect of AChEI therapy in stabilized patients is more likely to be found in those areas that are less involved by the disease process, such as the frontal ones, at least in mild and moderate stages of the disease (*33*).

The reasons underlying the heterogeneous responses of AD patients to long-term AChEI therapy, in terms of neuropsychologic and brain perfusion values, are not immediately clear, although wide variations in AChEI levels in cerebrospinal fluid were reported recently, suggesting variable central nervous system bioavailability (*34*). Carriage of at least 1 Apo E ϵ4 allele may be predictive of a poor response to AChEI therapy (*35*), but our results and those of a recent study with metrifonate (*36*) fail to confirm this.

In this study, patient stabilization cannot be ascribed entirely to the effect of AChEIs. In fact, the 2 subgroups were segregated on the basis of a literature-derived cutoff, and an appropriately wide untreated control group was not available. Therefore, the possibility that some patients with a stabilized course would have performed well after 1 y even without receiving AChEIs (i.e., a smoothed natural course of the disease) cannot be excluded. Another variable that was not addressed, but should be considered in interpreting our data, is the possible nonspecific rCBF effect of AChEIs (i.e., the placebo effect). However, such an effect has been estimated to disappear after 20 wk of therapy in AD patients undergoing AChEI therapy (*37*).

A positive effect on rCBF, albeit with some regional differences, was shown by ^{133}Xe quantitative rCBF measurement in 9 AD patients receiving tacrine therapy for 14 mo compared with untreated AD controls (*38*). The effects of short- to medium-term cholinergic treatment on rCBF in patients with AD have been reported. Staff et al. (*31*), in a retrospective study of 11 patients undergoing a second ^{99m}Tc-HMPAO SPECT examination after an average of 35 wk on donepezil therapy, reported an increase in global cerebral blood flow in 7 patients and a decrease in 4 patients. In a prospective open-label study, A. Venneri et al. (written communication, February 2001) reevaluated rCBF by ^{99m}Tc-HMPAO SPECT of 14 patients after 3 mo of regular rivastigmine administration and found that patients with a good neuropsychologic response to rivastigmine had increased rCBF in all regions, especially the frontal and parietal regions. Mega et al. (*39*) compared ^{18}F-FDG PET patterns in 12 AD patients treated with either metrifonate or donepezil for at least 4 wk. Cholinergic treatment resulted in a significant increase in metabolic activity in the anterior cingulate, the left dorsolateral frontal cortex, and the left supramarginal gyrus. Similar regions, but in the right hemisphere, were found to have a more preserved rCBF in AD patients receiving donepezil than in AD patients receiving a placebo after 1 y of therapy (*13*). However, in the latter study, the donepezil group was composed of only 15 patients, thus not permitting an investigation of the issue of heterogeneity of the cognitive decline among patients.

Clinical and neuropsychologic assessments are the current standard to evaluate the effect of AChEI treatment in AD, and the aim of this study was not to show an eventual, additional value of SPECT. Rather, our study was designed to investigate the rCBF (and, thus, neurophysiologic) correlates of long-term AChEI administration to AD patients. In fact, rCBF measurements are an indirect index of cerebral functional changes during the evolution of AD (*4*) and can investigate their topography. Clinical and neuropsychologic assessments imply their own typical limitations (i.e., to be strongly dependent on the examiner's expertise and the patient's motivation). In this context, the potential clinical values of SPECT examination of the rCBF deserves further attention because increasing evidence suggests that the integration of clinical and neuropsychologic data with functional neuroimaging may be used in the clinical setting to improve diagnostic power (*6*) and to assess the effect of therapy as well (*40*). Finally, prospective longitudinal SPECT studies of patients with mild cognitive impairment will probably be able to clarify the clinical role of SPECT in very early AD.

CONCLUSION

SPECT examination of the rCBF evolves in different ways in AD patients with cognitive stabilization during AChEI therapy compared with AD patients who worsen despite therapy. SPECT is widely available; on the basis of our data, its role in assessing brain function of AD patients receiving chronic cholinergic therapy deserves further consideration.

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