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Double-Blind Placebo-Controlled Study with Citicoline in APOE Genotyped Alzheimer's Disease Patients. Effects on Cognitive Performance, Brain Bioelectrical Activity and Cerebral Perfusion*

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SUMMARY

Cytidine 5'-diphosphocholine (citicoline) is a an endogenous intermediate in the biosynthesis of structural membrane phospholipids and brain acetylcholine. Citicoline has been extensively used for the treatment of neurodegenerative disorders associated with head trauma, stroke, brain aging, cerebrovascular pathology and Alzheimer's disease. In this study we have investigated the efficacy and safety of the treatment with citicoline versus placebo in patients with Alzheimer disease. Thirty patients (age = 73.0 ± 8.5 years; range = 57-87 years) with mild to moderate senile dementia (GDS: stages 3-6) of the Alzheimer type were included in a double-blind, randomized and placebo-controlled clinical trial. After a 2-week period of drug washout, patients were treated with i) placebo (n = 17; age = 73 ± 5 years) or ii) 1,000 mg/day of citicoline (n = 13; age = 76 ± 9 years) for 12 weeks (84 days). Examinations were done at baseline (T0) and after the 12 weeks of treatment (T12). As compared to placebo, citicoline improved cognitive performance in Alzheimer's disease patients with APOE E4 (ADAS: difference between groups = -3.2 ± 1.8 scores, $p < 0.05$; ADAS-cog: difference between groups = -2.3 ± 1.5, ns); and this improvement on cognition was more pronounced (ADAS, $p < 0.01$; ADAS-cog: difference between groups = -2.8 ± 1.3, $p < 0.06$) in patients with mild dementia (GDS < 5). Citicoline also increased cerebral blood flow velocities in comparison with placebo ($p < 0.05$) when transcranial Doppler recordings from both hemispheres were considered together, as well as diastolic velocity in the left middle cerebral artery ($p < 0.05$). Patients treated with citicoline showed an increase in the percentage of brain bioelectrical activity of α (occipital electrodes) and θ type (left side electrodes), accompanied by a decrease in relative delta activity particularly marked in the left temporal lobe. Significant differences with respect to placebo ($p < 0.05$) were observed for θ activity in several fronto-parieto-temporal electrodes of the left hemisphere. Treatment with citicoline tended to reduce serum IL-1β levels, mainly after 4 weeks of administration, with no modified blood histamine content. In addition, neither adverse side effects nor alterations in biological and hematological parameters were induced by citicoline. The present data indicate that citicoline (1,000 mg/day) is well tolerated and improves cognitive performance, cerebral blood perfusion and the brain bioelectrical activity pattern in AD patients. According to our results, it seems that citicoline might be a useful treatment in Alzheimer's disease, and that the efficacy of this compound is greater in patients with mild mental deterioration and/or bearing the ε4 allele of the APOE. ©1999 Prous Science. All rights reserved.

Key words: Citicoline - Alzheimer's disease - APOE genotype - Cognition - Brain activity

INTRODUCTION

Cytidine 5'-diphosphocholine (citicoline) is an endogenous intermediate involved in the biosynthetic pathway of structural membrane phospholipids, specially phosphatidylcholine (1-4). After its *per os* or parenteral administration citicoline releases the two main components of the molecule, cytidine and choline. The absorption of citicoline after oral intake is almost complete as its bioavailability by oral and intravenous administration is very similar. After absorption, citicoline is widely distributed in the body, crosses the blood-brain barrier and reaches the central nervous system (CNS), where it is incorporated into the phospholipid fraction of membranes and microsomes.

Citicoline activates the synthesis of neuronal membrane phospholipids and acetylcholine, increases cerebral metabolism, influences the activity of several neurotransmitters and metabolic factors into the brain, and modulates some neuroimmunotrophic mechanisms (1-14). Experimental studies demonstrate that citicoline increases noradrenaline, dopamine and other neurotransmitters into the CNS (5-8). In conditions of cerebral hypoxia and/or ischemia, citicoline promotes phospholipid synthesis and reduces the release of free fatty acids,

*This article is based on a study first reported in *Annals of Psychiatry* 1999, Volume 7: 331-52. ©1999 Prous Science.

improves cerebral glucose uptake and metabolism, exerts antiaggregatory and antiapoptotic effects, and reduces neuronal damage (9-12). It has also been demonstrated that citicoline reduces edema, phospholipase A_2 activation and the inhibition of ATPase activities induced by brain trauma (13, 14). In animal models of neurodegeneration induced by the injection of β-amyloid into the rat hippocampus, citicoline reverses learning and memory impairment, protects against neuronal loss, counteracts astroglial activation and the abnormal accumulation of MAP-2 in neuronal somata, and reduces the number of apoptotic neurons in a dose-dependent manner (9, 15-17). In addition, citicoline exerts procognitive effects in rodents, reverses amnesia induced by scopolamine, lesion of the nucleus basalis of Meynert or bromazepam, and ameliorates cognitive deficits in aged animals (18-20). All these data indicate that citicoline has neuroprotective and procognitive effects in several experimental conditions.

Various clinical studies demonstrate that citicoline is effective in the treatment of patients with brain trauma, stroke and chronic cerebrovascular disorders, vascular dementia and Alzheimer's disease, as well as in improving memory deficits in elderly nondemented people (6, 21-27). In old subjects, Suryani *et al.* (21) found that citicoline improves memory performance in some tasks (forward and backward digit span, logical story test and Bali picture memory test) and reduces memory and physical complaints. These positive effects of citicoline on senile cognitive impairment were recently corroborated by Spiers *et al.* (22) and by Álvarez *et al.* (23). On the other hand, clinical trials conducted with stroke, vascular dementia and Alzheimer's patients indicate that citicoline improves neurologic dysfunctions and cognitive performance, and exerts vasoregulatory, electrophysiologic and immunomodulatory effects (24-28).

According to results of the toxicology tests performed, citicoline if a safe and well-tolerated drug, lacking relevant systemic cholinergic effects. Severe adverse events were not reported in any of the series of patients treated with citicoline. The pharmacological profile and the potential mechanisms of action of citicoline suggest this compound might be a first choice treatment for neurodegenerative disorders such as Alzheimer's disease.

Objectives of the present study included: i) evaluating the efficacy of citicoline (1,000 mg/day) *vs.* placebo on cognitive performance, brain bioelectrical activity and cerebral hemodynamics in patients with Alzheimer's disease of mild to moderate severity; ii) determining the effects of citicoline on circulating levels of interleukin-1β (IL-1β) and histamine as an indicator of its potential neuroimmune activity; and iii) assessing the safety of citicoline treatment, monitoring biologic and biochemical parameters and recording adverse events.

MATERIALS AND METHODS

Patients

Thirty outpatients of both sexes (age = 74.0 ± 8.5 years; range = 57-87 years) with mild to moderate senile dementia of the Alzheimer type were selected according to the following criteria: age of at least 50 years, a diagnosis of probable Alzheimer's disease as defined by the ICD10 (29), DSM-IV (30) and NICDS-ADRDA (31) criteria, a score of 14 to 26 (inclusive) in the mini mental state examination (MMSE) of Folstein (32), and a stage of 3 to 6 (inclusive) in the global deterioration scale (GDS) (33). Patients were excluded if they had another disease or required concurrent medications known to affect the central nervous system. Subjects with scores of 16 or higher in the 21-item Hamilton rating scale for depression (34) were also excluded. Each patient had a caregiver to ensure the compliance of the protocol. Written informed consent was obtained from the patient (or his/her legal representative) and the caregiver. The study protocol was approved by the Spanish Ministry of Health and conducted according to guidelines of the Spanish Law on Good Clinical Practices and its application to the conduction of clinical trials (R.D. 561/1993).

Study design and treatment

This was a pilot, double-blind, placebo-controlled and randomized clinical trial with two parallel groups. Patients were randomly assigned to two different treatment groups in a noncompensatory manner (different number of subjects in each group). After a 2-week period of drug washout, patients were treated with i) placebo (n = 17; 11 women/6 men; age = 73 ± 5 years; age range = 64-79 years) or ii) 1,000 mg/day of citicoline (n = 13; 11 women/2 men; age = 76 ± 9 years; age range: 57-87 years) for 12 weeks (84 days). Citicoline (1,000 mg/4 cc/day) was administered orally once a day. Placebo (4 cc tridistilled water) was given orally every day during the 14-day washout period (all patients), the 12 weeks of treatment (placebo group only), and the 1-week post-treatment discontinuation phase (all patients). Safety and efficacy evaluations were done at baseline (T0, before the double-blind phase) and after 12 weeks of active treatment (T12). Treatment efficacy was also evaluated as a function of the apolipoprotein E (APOE) genotype. The number of patients with APOE 2/3, 3/3, 3/4 and 4/4 genotypes were 1, 6, 6 and 4 cases, respectively, in the placebo group, and 0, 3, 6 and 4 cases, respectively, in the citicoline group.

Outcome measures: Efficacy and safety

Efficacy was assessed according to primary and secondary measures. The cognitive-function subscale of the Alzheimer's disease assessment scale (ADAS) (35) and the clinical interview based impression of change (CIBIC) (36) were used as primary outcome measures.

The cognitive subscale of the ADAS includes assessment of word recall, naming of common objects and fingers, the ability to follow simple commands, constructional (copying figures) and ideational (addressing a letter) praxis, orientation, word recognition memory, spoken language, comprehension of spoken language, word finding and recall of test instructions. The score range of the subscale varies from 0 points (no cognitive deficit) to 70 points (severe cognitive deficit). The CIBIC is a global change measure which reflects data gathered from an interview conducted by a skilled and experienced physician or neuropsychologist familiar with the manifestations of dementia. The format for scoring provides for symmetrical improvement or worsening using a 7-point ordinal scale (*e.g.*, 4, no change; 5, 6, 7, increasing degrees of deterioration; 3, 2, 1, increasing degrees of improvement).

Secondary outcome measures were other psychometric tests (ADAS: total score, memory subtest, and noncognitive subscale; MMSE; trail making test: performance time and total score); brain bioelectrical activity pattern; blood flow hemodynamics in the middle cerebral artery (MCA); and circulating levels of IL-1β and histamine.

Brain bioelectrical activity was assessed with computerized EEG spectral analysis and topographic brain mapping. EEG and brain mapping recordings were obtained by using previously described procedures (37, 38). Data were normalized and relative power (%) was used as reference parameter. The following frequency bands were studied: δ (0.5-3.5 Hz), θ (4-7.5 Hz), α (8-11.5 Hz) and β (12-15.5 Hz).

Hemodynamic changes in the middle cerebral artery were evaluated following the same protocol used before (38, 39). Blood flow parameters studied in the right and left MCA were average mean (Mv), systolic (Sv) and diastolic (Dv) flow velocities, pulsatility index (PI = |Sv-Dv|/Mv), resistance index (RI = |Sv-Dv|/Sv), and the effective pulsatility range (EPR = Mv-|Sv-Dv|).

Histamine and IL-1β levels were determined by HPLC and ELISA, respectively, as in previous studies (28). Methods for the identification of the APOE polymorphism were also described in other publications of our group (40).

Safety was evaluated by using physical examination, vital signs, laboratory tests (hematology, biochemistry), EKG and recording of adverse events.

Statistics

In order to evaluate the efficacy of citicoline, changes from baseline (T0) were analyzed for all the variables studied after treatment (T12). Parametric (*t*-test) and nonparametric (Wilcoxon) tests were used to compare paired data obtained before and after treatment in each group (intragroup analysis). Analysis of covariance was used to compare treatment groups (intergroup analysis), with the value from T12 (post-treatment) as the dependent variable and baseline (T0) scores as covariates. Statistical analyses of the treatment effect were performed in the total sample, in the subgroup of patients with some APOE ε4 allele (APOE4), and in patients with APOE4 and mild dementia (GDS < 5). Probability values lower than 0.05 were considered significant.

In the case of the CIBIC scale, both direct T12 scores, which reflect the change with respect to T0, and scores of change from baseline were compared. Distribution of frequencies by group was also analyzed.

RESULTS

Effects of citicoline on cognitive performance

Mean scores obtained by the two groups (citicoline and placebo) in psychometric tests and the CIBIC at baseline (T0) and after 12 weeks of treatment (T12) are presented in Table 1. Average values of change from baseline for each test are also reflected. Differences with respect to baseline tended to be opposite in the two treatment groups, indicating that citicoline reverses or ameliorates the worsening in cognitive performance observed in subjects treated with placebo.

Intragroup analysis showed that CIBIC scores increased (worsening) significantly ($p < 0.05$) in the placebo group and decreased (improvement) in a nonsignificant manner in subjects treated with citicoline.

When the total sample was considered, differences between groups in CIBIC and psychometric scores did not reach significant values, although trends of change favor citicoline. However, as compared with placebo, citicoline induced a significant improvement in ADAS performance (difference between groups: -3.23 ± 1.8 points; $p < 0.05$) in patients bearing the ε4 allele of the APOE (APOE4). Citicoline also improved ADAS scores with respect to placebo (difference between groups: -3.1 ± 0.91 points; $p < 0.01$) in APOE4 patients with mild cognitive deterioration (GDS < 5).

In comparison with placebo, citicoline improved ADAS-cog scores as well, but this improvement was not significant neither for the total sample (difference between groups: -1.58 ± 1.34 points) nor for the subgroup of APOE4 patients (difference between groups: -2.31 ± 1.52 points), and reached almost significant values in APOE4 and GDS < 5 patients (difference between groups: -2.83 ± 1.35 points; $p < 0.06$). Changes from baseline in ADAS and ADAS-cog observed in both treatment groups and in the different samples studied are shown in Figure 1.

Effects of citicoline on brain hemodynamics

Data on brain hemodynamic parameters are shown in Table 2. These results reflect a decrease in cerebral blood flow velocities and in the effective pulsatility range

TABLE 1. Effects of citicoline on cognitive performance in Alzheimer's disease patients.

Variable (scoring range)	Placebo n	Placebo Baseline (T0)	Placebo T12	Placebo Change	Citicoline n	Citicoline Baseline (T0)	Citicoline T12	Citicoline Change
Primary Variables								
ADAS (0-120)	16	36.75 ± 4.89 (26.3; 47.1)	37.06 ± 5.13 (26.1; 48.0)	0.31 ± 0.99 (−1.7; 2.4)	12	36.33 ± 5.76 (23.6; 49.0)	35.16 ± 6.03 21.8; 48.4)	−1.16 ± 1.35 (−4.1; 1.8)
ADAS-cog (0-70)	16	31.81 ± 4.35 (22.5; 41.0)	32.96 ± 4.51 (22.4; 41.6)	0.25 ± 0.80 (-1.4; 1.9)	12	31.08 ± 5.14 (19.7; 42.4)	29.75 ± 5.31 (18.0; 41.4)	−1.33 ± 1.12 (−3.8; 1.1)
CIBIC (1-7)								
Mean score	17	4.0	4.35 ±0.14* (4.0; 4.6)	0.35 ± 0.14 (0.04; 0.6)	13	4.0	3.84 ± 0.31 (3.1; 4.5)	−0.15 ± 0.31 (−0.8; 0.5)
Secondary variables								
ADAS-C (0-48)	16	17.00 ± 2.70 (11.2; 22.7)	16.93 ± 2.90 (10.7; 23.1)	−0.06 ± 0.60 (−1.3; 1.2)	12	16.58 ± 3.46 (8.9; 24.2)	15.58 ± 3.48 (7.9; 23.2)	−1.00 ± 0.82 (−2.8; 0.8)
ADAS-M (0-22)	16	14.81 ± 1.68 (11.2; 18.4)	15.12 ± 1.71 (11.4; 18.7)	0.31 ± 0.51 (−0.7; 1.4)	12	14.50 ± 1.76 (10.6; 18.3)	14.16 ± 1.88 (10.0; 18.3)	-0.33 ± 0.54 (−1.5; 0.8)
ADAS-NC (0-50)	16	4.93 ± 0.76 (3.3; 6.5)	5.00 ± 0.86 (3.1; 6.8)	0.06 ± 0.58 (-1.1; 1.3)	12	5.25 ± 1.03 (2.9; 7.5)	5.41 ± 1.17 (2.8; 8.0)	0.16 ± 0.56 (−1.0; 1.4)
MMSE (0-30)	16	19.25 ± 1.05 (17.1; 21.4)	19.87 ± 0.98 (17.7; 21.9)	0.62 ± 0.44 (-0.3; 1.5)	12	19.33 ± 1.13 (16.8; 21.8)	19.5 ± 1.19 (16.8; 22.1)	0.16 ± 0.54 (−1.0; 1.3)
TMT (0-300)	16	177.0 ± 19.2 (136.0; 218.0)	187.2 ± 21.8 (140.6; 233.8)	10.1 ± 18.0 (-28.8; 48.6)	12	184.5 ± 24.0 (131.4; 237.5)	187.0 ± 21.8 (128.7; 245.2)	2.50 ± 7.63 (−14.4; 19.4)
TMP (0-25)	16	14.93 ± 2.77 (9.0; 20.8)	15.93 ± 2.74 (10.0; 21.7)	1.00 ± 0.90 (−0.9; 2.9)	12	12.83 ± 3.53 (5.0; 20.6)	13.58 ± 3.42 (6.05; 21.1)	0.75 ± 0.42 (−0.1; 1.6)

Scores: mean ± SEM (CI 95%). Change: Difference between T12 and T0 scores. Subjects without complete psychometric assessment (one in each group) were not considered in the statistical analysis. ADAS: Alzheimer's disease assessment scale. ADAS-cog: cognitive subscale of the ADAS. ADAS-M: memory subtest of the ADAS. ADAS-C: ADAS-cog minus ADAS-M. ADAS-NC: noncognitive subscale of the ADAS. CIBIC: clinical interview based of change. MMSE: mini mental state examination. TMT: trail making test, performance time (seconds). TMP: total scoring in the TMT. *$p < 0.05$ vs. baseline.

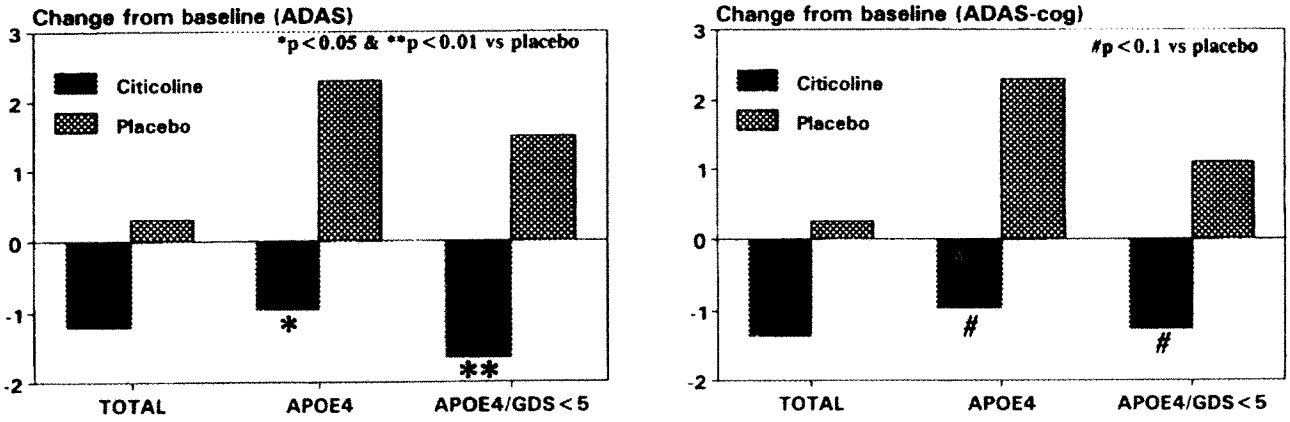

FIG. 1. Effect of citicoline on cognition in patients with Alzheimer's disease. Changes from baseline in ADAS and ADAS-cog performance were evaluated in the whole sample, in patients with the epsilon4 allele of the apolipoprotein E (APOE4), and in patients with APOE4 and mild cognitive impairment (GDS < 5).

(EPR) in right and left middle cerebral arteries from patients of the placebo group, as well as an increase (or no decrease) of these values in patients treated with citicoline, especially in the left middle cerebral artery.

As compared to baseline, after treatment with placebo, we observed a significant decrease in diastolic velocities in the left middle cerebral artery ($p < 0.05$) and in the right ($p < 0.01$), a reduction in mean velocity ($p < 0,05$) and EPR ($p < 0.01$), and an increase in the resistance index ($p < 0.05$) in the right side. Although variations in flow velocities are opposite to those found in the placebo group, citicoline did not induce any significant change from baseline in hemodynamic parameters (Figs. 2-4).

TABLE 2. Effects of citicoline on brain hemodynamics in patients with Alzheimer's disease.

		Placebo				Citicoline		
Artery: Variable	*n*	Baseline (T0)	T12	Change	*n*	Baseline (T0)	T12	Change
LMCA:								
Mv	15	39.05 ± 1.59	36.76 ± 1.81	−2.28 ± 1.54	10	35.20 ± 3.18	37.08 ± 3.21	1.88 ± 2.48
		(35.6; 42.4)	(32.8; 40.6)	(−5.5; 1.0)		(27.9; 42.4)	(29.8; 44.3)	(−3.7; 7.4)
Sv	15	60.66 ± 2.33	58.26 ± 2.72	−2.40 ± 2.35	10	55.72 ± 5.05	60.34 ± 5.16	4.61 ± 5.04
		(55.6; 65.6)	(52.4; 64.1)			(44.2; 67.1)	(48.6; 72.0)	(−6.7; 16.0)
Dv	15	(24.75 ± 1.20)	22.48 ± 1.31*	−2.24 ± 1.10	10	21.12 ± 1.96	22.05 ± 2.16	0.93 ± 1.34#
		(21.9; 27.5)	(19.6; 25.3)	(−4.6; 0.1)		(1.6.6; 25.5)	(17.1; 26.9)	(−2.1; 3.9)
EPR	15	3.13 ± 1.66	0.98 ± 1.47	−2.15 ± 1.41	10	0.60 ± 1.42	−1.20 ± 1.96	−1.8 ± 1.96
		(0.4; 6.7)	(−2.1; 4.1)	(−5.1; 0.8)		(−2.6; 3.8)	(-5.6; 3.2)	(−6.2; 1.2)
PI	15	0.92 ± 0.039	0.98 ± 0.039	0.053 ± 0.037	10	0.98 ± 0.042	1.04 ± 0.052	0.061 ± 0.050
		(0.84; 1.01)	(0.89; 1.06)	(−0.02; 0.13)		(0.91; 1.05)	(0.92; 1.16)	−0.05; 0.17)
RI	15	0.59 ± 0.014	0.61 ± 0.014	0.022 ± 0.014	10	0.61 ± 0.016	0.63 ± 0.018	0.018 ± 0.019
		(0.55; 0.62)	(0.58; 0.64)	(−0.008; 0.05)		(0-57; 0.65)	(0.59; 0.67)	(−0.02; 0.06)
RMCA:								
Mv	17	39.44 ± 2.24	35.76 ± 1.92*	−3.69 ± 1.41	12	37.75 ± 3.23	37.14 ± 3.18	−0.60 ± 1.92
		(34.6; 44.1)	(31.6; 39.8)	(−6.7; -0.7)		(32.7; 42.9)	(30.1; 44.1)	(−4.8; 3.6)
Sv	17	62.10 ± 4.08	58.36 ± 3.64	−3.73 ± 1.99	12	59.25 ± 5.09	60.62 ± 4.77	1.37 ± 4.15
		(53.4; 70.8)	(50.6; 66.1)	(−7.9; 0.4)		(48.0; 70.4)	(50.1; 71.1)	(−7.7; 10.5)
Dv	17	24.61 ± 1.42	21.20 ± 1.14**	−3.41 ± 1.13	12	23.28 ± 2.30	21.95 ± 2.12	−1.22 ± 1.08
		(21.5; 27.6)	(18.7; 23.6)	(−5.8; -1.0)		(18.1; 28.2)	(17.2; 26.6)	(−3.6; 1.1)
EPR	17	1.95 ± 1.68	−1.41 ± 1.87**	−3.36 ± 1.26	12	1.12 ± 2.33	−1.52 ± 2.12	−2.64 ± 1.96
		(−1.6; 5.5)	(−5.3; 2.5)	(−6.0; −0.7)		(−4.0; 6.2)	(-6.1; 3.1)	(−6.9; 1.6)
PI	17	0.95 ± 0.039	1.03 ± 0.053	0.084 ±0.042	12	0.97 ± 0.055	1.06 ± 0.061	0.092 ± 0.049
		(0.86; 1.03)	(0.92; 1.14)	(−0.006; 0.17)		(0.85; 1.09)	(0.93; 1.20)	(−0.01; 0.2)
RI	17	0.59 ± 0.015	0.62 ± 0.017*	0.029 ± 0.013	12	0.60 ± 0.021	0.63 ± 0.018	0.029 ± 0.017
		(0.56; 0.62)	(0.59; 0.66)	(0.001; 0.05)		(0.56; 0.65)	(0.59; 0.67)	(−0.008; 0.06)

LMCA: left middle cerebral artery; RMCA: right middle cerebral artery. Mv: mean velocity. Sv: systolic velocity. Dv: diastolic velocity. EPR: effective pulsatility range. PI: pulsatility index. RI: resistance index. Variations in number (*n*) are due to difficulties in obtaining TCD recordings in some patients. Scores: mean ± SEM (CI 95%). *$p < 0.05$. **$p < 0.01$ *vs.* basal. #$p < 0.05$ *vs.* placebo.

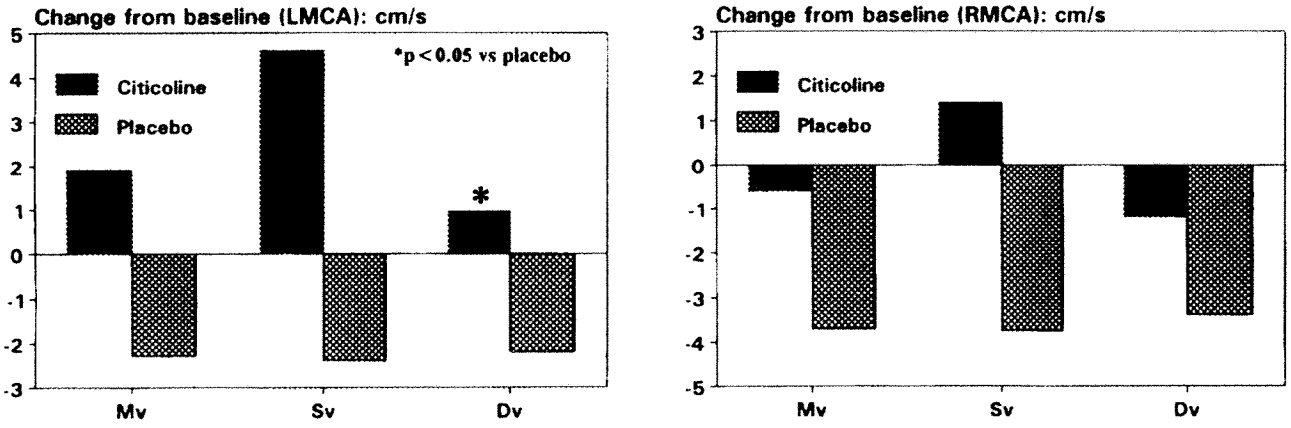

FIG. 2. Effect of citicoline on brain hemodynamics in Alzheimer's disease patients. Changes from baseline in mean (Mv), systolic (Sv) and diastolic (Dv) blood flow velocities recorded in left (LMCA) and right (RMCA) middle cerebral arteries are shown.

When data of the two MCA were analyzed together, citicoline induced a significant ($p < 0.05$) increase in mean, systolic and diastolic velocities with respect to placebo (data not shown). Changes from baseline in left middle cerebral artery diastolic velocity were also different in the two groups (difference between groups: 3.4 ± 1.7 cm/s; $p < 0.05$). The same trend of change was found for all the flow velocities in the two MCA (Figs. 2-4).

Effects of citicoline on brain bioelectrical activity

Brain bioelectrical activity of the alpha type showed a decrease from baseline in the placebo group and an increase after treatment with citicoline. Differences between groups for relative α power were almost statistically significant in left (O1: 4.86 ± 2.88; $p < 0.1$), right (O2: 5.29 ± 3.04; $p < 0.1$) and central occipital electrodes (5.03 ± 2.71; $p < 0.1$) (Table 3, Figs. 5, 6).

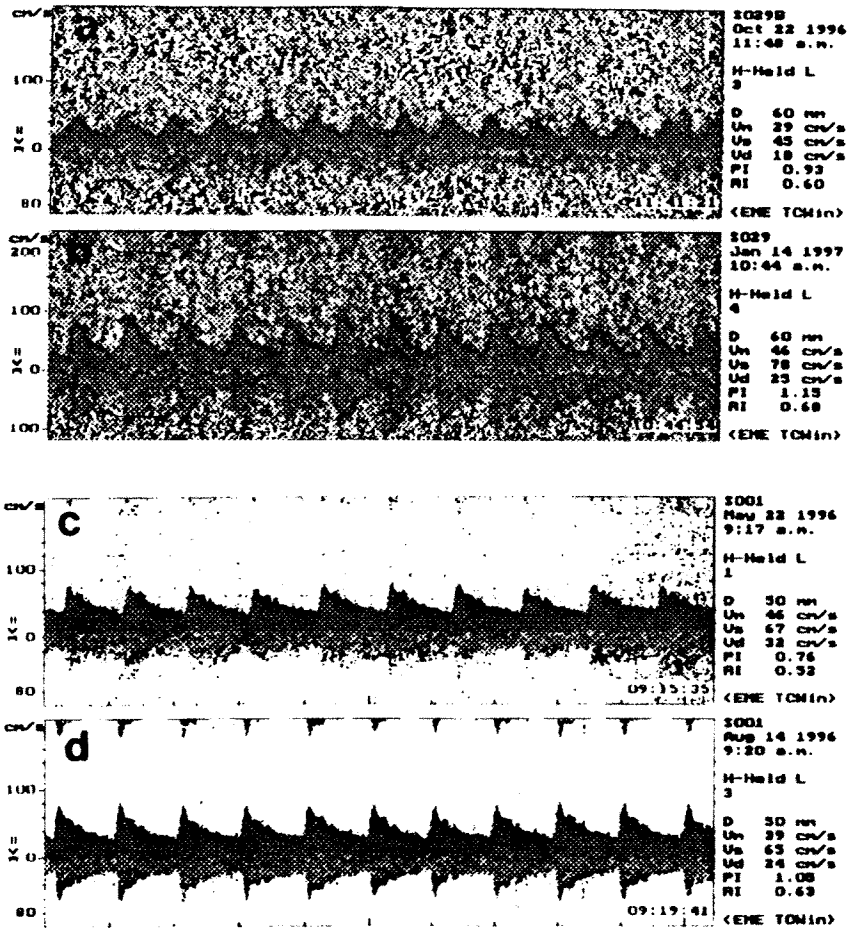

FIG. 3. Spectral analysis of the left middle cerebral artery in a patient treated with citicoline (top recordings) and a patient from the placebo group (bottom recordings), obtained at baseline (a, c) and 12 weeks after treatment (b, d). Note the increase in blood flow velocities induced by citicoline and the decrease in the case of placebo.

Changes from baseline in β activity were opposite to that observed for α activity in all the occipital electrodes, and differences between groups reached significant values in O2 (-3.12 ± 1.38; $p < 0.05$) (Table 3, Fig. 6).

A generalized increase in θ activity power was found in the citicoline group. As compared with placebo, this increase in theta frequencies after citicoline treatment was statistically significant ($p < 0.05$) in most of the electrodes of the left hemisphere of the brain (C3: 6.8 ± 2.9; F3: 9.4 ± 3.7; F7: 11.1 ± 3.2; FP1: 8.1 ± 3.7; P3: 6.1 ± 2.6; T3: 8.2 ± 3.6; and T5: 6.9 ± 4.3) (Table 4, Fig. 7). On the contrary, slow δ activity showed a global decrease in subjects treated with citicoline. This decrease in δ power induced by citicoline was more pronounced in the left hemisphere, and reached significant values in T3 (change from baseline: -10.96 ± 3.94; $p < 0.05$). Changes in δ activity were not significantly different between treatment groups (Table 4, Fig. 7).

Effects of citicoline on IL-1β and histamine levels

Plasma IL-1β levels decreased after citicoline treatment, mainly at the 4-week period, and increased in patients receiving placebo (Table 5). However, statistically significant changes were not found in the intragroup analyses or in the between-group comparisons. Variations in histamine concentrations were not significant in the two therapeutic groups (Table 6). IL-1β and histamine levels were consistently, but not significantly, lower in the placebo group than in the citicoline group during the study.

Adverse events and safety biology parameters

No severe adverse events were observed in this study. In the group treated with citicoline, three mild adverse events were reported (a cold, constipation and coughing) none of which were related to drug intake; while five mild adverse events were recorded in the placebo group (orthostatic hypotension, anxiety disorder, cranial trauma, headache and a cold).

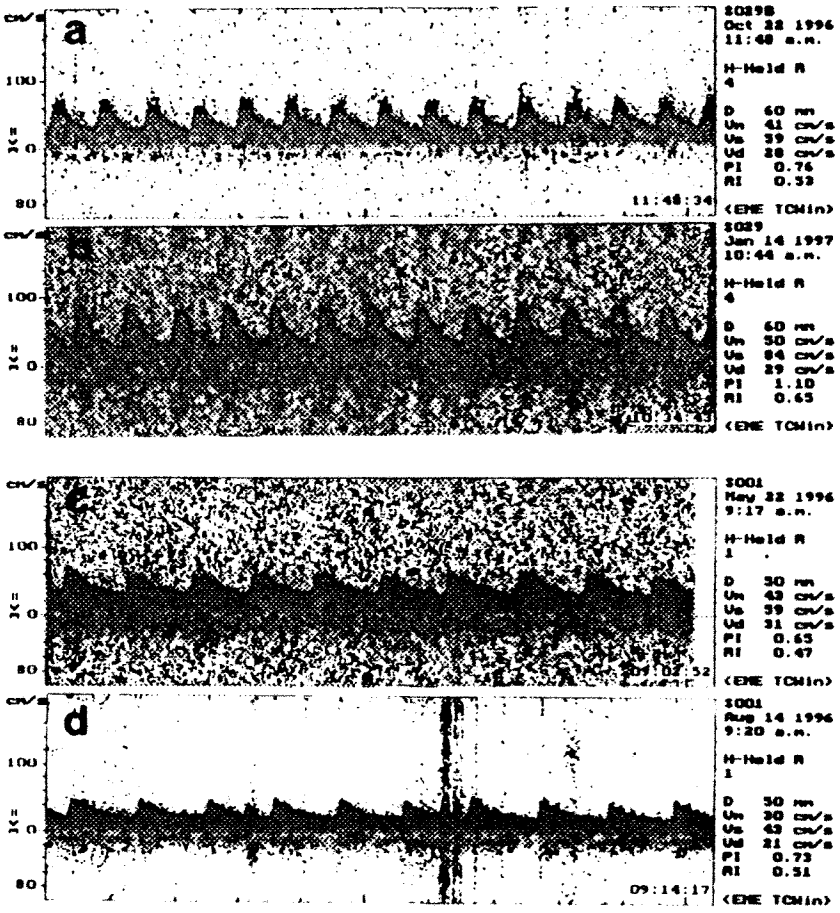

FIG. 4. Spectral analysis of the right middle cerebral artery in patients treated with citicoline (top pictures) or placebo (bottom pictures). Recordings were obtained at baseline (a, c) and after 12 weeks of treatment (b, d). Note that citicoline increases blood flow velocities, whereas placebo reduces them dramatically.

TABLE 3. Effects of citicoline on occipital alpha and beta bioelectrical activity in Alzheimer's disease patients.

		Placebo				Citicoline		
Activity: Electrode	*n*	Baseline (T0)	T12	Change	*n*	Baseline (T0)	T12	Change
α								
O1	17	35.05 ± 3.95	30.87 ± 3.22	−4.18 ± 2.64	13	25.40 ± 4.43	26.08 ± 4.60	0.68 ± 1.14
		(26.6; 43.4)	(24.0; 37.6)	(−9.7; 1.4)		(15.7; 35.0)	(16.0; 36.1)	(−1.8; 3.1)
O2	17	40.61 ± 4.92	37.61 ± 4.32	−2.99 ± 2.26	13	29.82 ± 5.55	32.12 ± 5.57	2.30 ± 1.82
		(30.1; 51.0)	(28.4; 46.7)	(−7.7; 1.8)		(17.7; 41.9)	(19.9; 44.2)	(−1.6; 6.2)
OZ	17	38.08 ± 4.44	34.69 ± 3.68	−3.39 ± 2.42	13	27.60 ± 4.94	29.23 ± 4.98	1.62 ± 1.22
		(28.6; 47.5)	(26.8; 42.4)	(−8.5; 1.7)		(16.8; 38.3)	(18.3; 40.0)	(−1.0; 4.3)
β								
O1	17	7.98 ± 1.24	10.66 ± 1.44	2.68 ± 0.87*	13	9.60 ± 1.60	9.34 ± 1.23	−0.26 ± 1.25
		(5.3; 10.6)	(7.6; 13.7)	(0.8; 4.5)		(6.1; 13.1)	(6.6; 12.0)	(−0.3; 2.4)
O2	17	7.17 ± 0.99	9.43 ± 1.26	2.25 ± 0.89*	13	9.59 ± 1.66	8.72 ± 1.28	−0.86 ± 1.06#
		(5.0; 9.2)	(6.7; 12.1)	(0.3; 4.1)		(5.9; 13.2)	(5.9; 11.5)	(−3.1; 1.4)
OZ	17	7.65 ± 1.06	10.04 ± 1.33	2.38 ± 0.88*	13	9.60 ± 1.60	0.93 ± 1.22	0.57 ± 1.12
		(5.4; 9.9)	(7.2; 12.8)	(0.5; 4.2)		(6.1; 13.1)	(6.3; 11.7)	(−3.0; 1.8)

Occipital electrodes: left (O1), right (O2) and central (OZ). Scores: mean ± SEM (CI 95%). α and β activity relative power (%). *$p < 0.05$ *vs.* basal. #$p < 0.05$ *vs.* placebo.

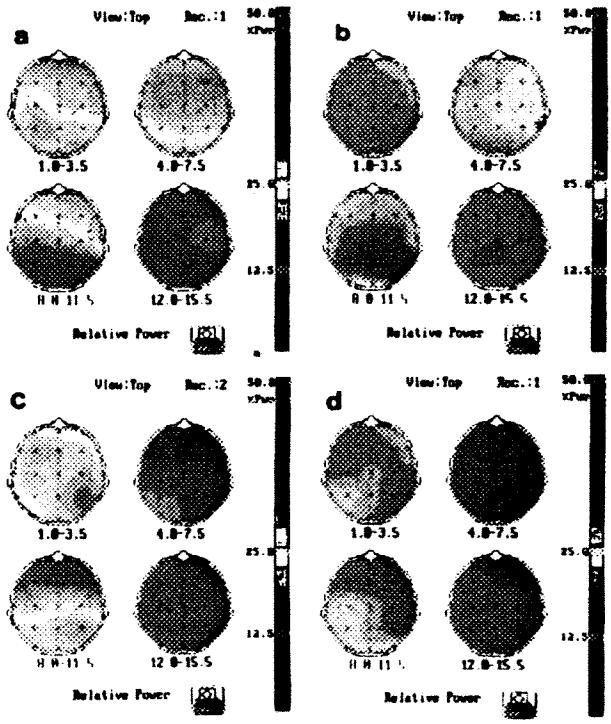

FIG. 5. Topographic brain maps from patients treated with citicoline (top) or placebo (bottom), obtained in basal conditions (left) and after treatment (right). It can be observed how citicoline induces a decrease in slow (δ and θ) activity and an increase in α frequencies (a, b), while placebo increases theta and reduces α relative power (c, d).

During the clinical trial, no significant changes were observed in the values of arterial blood pressure, heart rate, body temperature and body weight in subjects treated with placebo or in those receiving citicoline. Alterations in electrocardiogram recordings or abnormalities in laboratory tests related to treatment intake were not detected.

DISCUSSION

Results of the present study demonstrate that citicoline improves cognitive performance in patients with mild to moderate Alzheimer's disease, especially in patients with APOE4 and GDS < 5 (initial stages of the disease). As compared with placebo, citicoline induced a significant improvement in the ADAS, as well as an almost significant improvement in the ADAS-cog. These effects were more pronounced in APOE4 and GDS < 5 patients. The clinical impression of change, as assessed with the CIBIC scale, showed a significant worsening in the placebo group and a positive trend in the citicoline group, but differences between groups did not reach statistical significance. In general, it was observed that performance in psychometric tests tended to improve in patients treated with citicoline and to worsen in the placebo group after 12 weeks of treatment. These data are in agreement with previous studies demonstrating that citicoline improves mental functioning in elderly nondemented subjects and in patients with senile dementia of the vascular or the Alzheimer type (21-27).

There are some facts that need to be taken into account for the evaluation of the cognitive effects of citicoline in the present study. Firstly, all the patients selected for this study took citicoline during several months before selection. Since the procognitive activity of this drug seems to last for at least 1 month after withdrawal (data not shown), it is possible that both cognitive worsening in the placebo group and cognitive improvement in the group of active treatment might appear attenuated. In addition, the efficacy of citicoline was evaluated in a

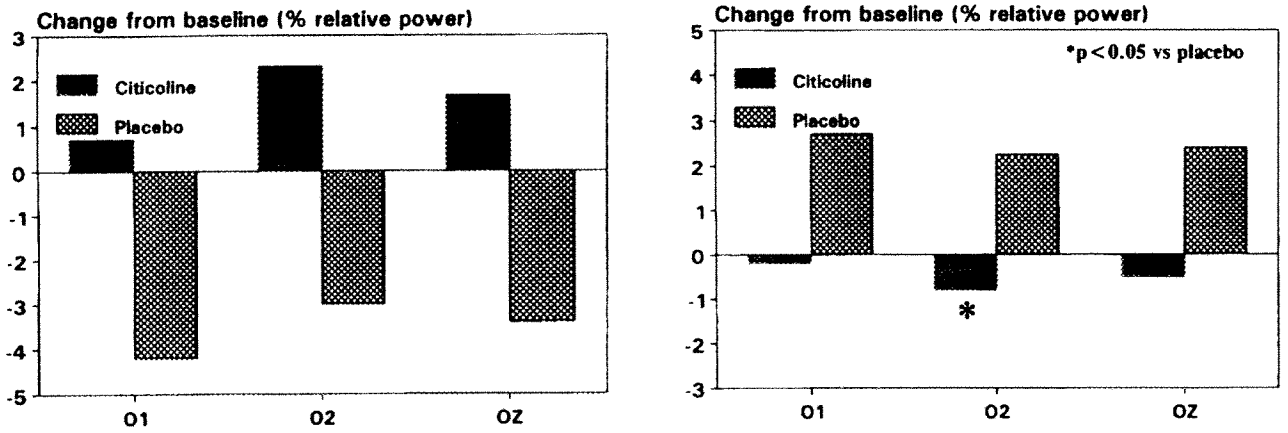

FIG. 6. Effects of citicoline on brain bioelectrical activity of the α (A) and β (B) type in Alzheimer's disease. In comparison with baseline recordings, it is observed that occipital (O1, O2, OZ) α activity increases in patients treated with citicoline and decreases in those of the placebo group. Changes in beta activity are opposite to those found for α frequencies.

TABLE 4. Effect of citicoline on θ and δ bioelectrical activity in Alzheimer's disease patients.

		Placebo				Citicoline		
Activity: electrode	*n*	Baseline (T0)	T12	Change	*n*	Baseline (T0)	T12	Change
θ								
F3	17	29.92 ± 2.74 (24.1; 35.7)	30.35 ± 3.13 (23.7; 36.9)	0.42 ± 2.10 (-4.0; 4.8)	13	24.76 ± 2.68 (18.9; 30.6)	34.54 ± 5.16** (23.3; 45.8)	9.78 ± 3.20# (2.7; 16.7)
P3	17	25.65 ± 2.83 (19.6; 31.6)	27.23 ± 2.79 (21.3; 33.1)	1.57 ± 1.36 (-1.3; 4.4)	13	23.05 ± 2.85 (16.8; 29.2)	30.69 ± 4.34** (21.2; 40.1)	7.63 ± 2.30# (2.4; 12.8)
T3	17	29.02 ± 3.19 (22.2; 35.7)	27.53 ± 4.20 (18.6; 36.4)	-1.48 ± 2.41 (-6.6; 3.6)	13	20.56 ± 2.34 (15.4; 25.6)	27.23 ± 4.34* (17.7; 36.7)	6.66 ± 2.58# (1.0; 12.3)
δ								
F3	17	21.72 ± 3.07 (15.1; 28.2)	15.84 ±2.00 (11.5; 20.0)	-5.87 ± 3.14 (-12.5; 0.7)	13	26.85 ± 3.85 (17.4; 34.2)	17.72 ± 2.33 (12.6; 22.8)	-8.13 ± 4.85 (-18.7; 2.4)
P3	17	20.30 ± 3.24 (13.4; 27.1)	16.04 ± 2.36 (11.0; 21.0)	-4.25 ± 3.62 (-11.9; 3.4)	13	23.99 ± 4.04 (15.1; 32.7)	19.26 ± 2.24 (14.3; 24.1)	-4.73 ± 3.63 (-12.6; 3.1)
T3	17	22.32 ± 3.64 (14.5; 30.0)	15.71 ± 3.39 (8.5; 22.9)	-6.61 ± 4.65 (-16.4; 3.2)	13	28.56 ± 5.08 (17.4; 39.6)	17.60 ± 2.59* (11.9; 23.2)	-10.96 ± 3.94 (-19.5; -2.3)

Left frontal (F3), parietal (P3) and temporal (T3) electrodes. Scores: mean ± SEM (CI 95%). θ and δ activity relative power (%). *$p < 0.05$ and **$p < 0.01$ *vs.* basal. #$p < 0.05$ *vs.* placebo.

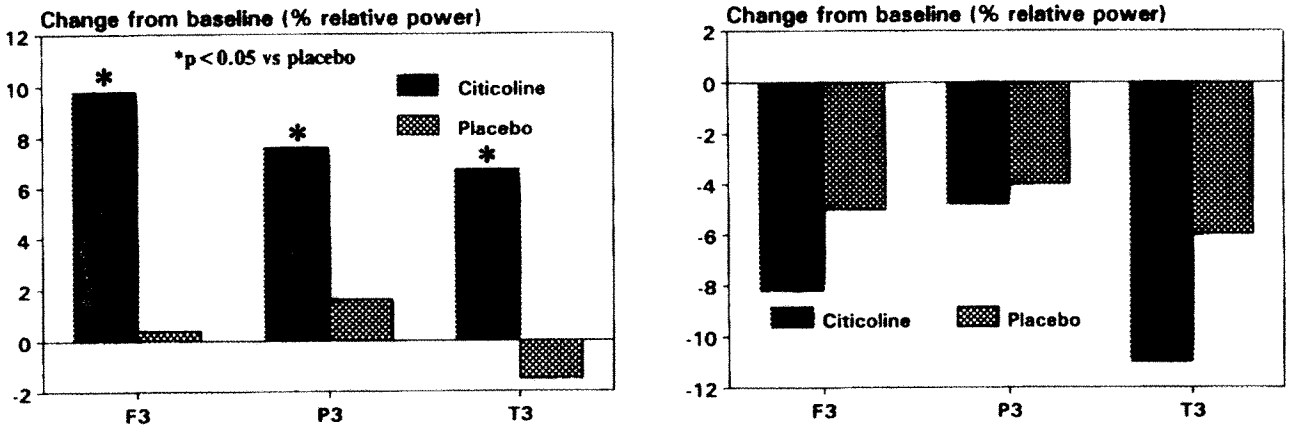

FIG. 7. Effects of citicoline on brain bioelectrical activity of θ (A) and δ (B) type in Alzheimer's disease patients. An increase of θ activity in electrodes of the left hemisphere and a decrease in relative δ power are evident after treatment with citicoline.

reduced sample (n = 12-13) owing to the fact that most of the patients were assigned to the placebo group. Therefore, it is likely that the effect of citicoline on cognition in Alzheimer's disease patients may be higher than that observed in this clinical trial. In a previous study (27) we found the highest procognitive action of citicoline in early onset Alzheimer's disease patients. This partial evaluation was not performed in the present study because of the small number of subjects treated with citicoline.

A very interesting aspect of our results is that the improvement in clinical and psychometric parameters induced by citicoline was higher in patients bearing the allele ε4 of the APOE. Nowadays, it is widely accepted that the APOE genotype may be useful in predicting drug response in Alzheimer's disease therapy. In previous studies it was found that the rate of responders to acetylcholinesterase inhibitors (AChE) such as tacrine was higher among non-ε4 bearers while the higher frequency of responders to other neuroprotective drugs such as S12024 was observed in APOE ε4 bearers (41, 42). The inverse relation described between the number of copies of the ε4 allele and the brain choline acetyltransferase activity in Alzheimer's disease might explain the influence of the APOE genotype on the clinical response to AChE inhibitors (42). According to these investigations, it seems reasonable to believe that citicoline exerts its neuroprotective activity through noncholinergic mechanisms. It is true that the best results were obtained in mild dementia patients (early stages of the disease), in which

TABLE 5. Effect of citicoline on plasma interleukin-1β levels in Alzheimer's disease.

		Placebo				Citicoline		
Parameter	*n*	Baseline (T0)	2 hours	Change	*n*	Baseline (T0)	2 hours	Change
IL-1β (pg/ml)	17	63.84 ± 11.40 (39.6; 88.0)	79.57 ± 11.93 (54.2; 104.8)	15.72 ± 12.19 (−10.1; 41.5)	13	94.61 ± 14.3 (63.3; 125.8)	91.49 ± 14.3 (61.8; 121.1)	−3.12 ± 19.55 (−45.7; 39.4)
Parameter	*n*	Baseline (T0)	T4	Change	*n*	Baseline (T0)	T4	Change
IL-1β (pg/ml)	17	63.84 ± 11.40 (39.6; 88.0)	76.00 ± 9.33 (56.2; 95.8)	12.16 ± 15.55 (−20.8; 45.1)	13	94.61 ± 14.3 (63.3; 125.8)	66.87 ± 14.12 (36.1; 97.6)	−27.73 ± 23.55 (−79.0; 23.5)
Parameter	*n*	Baseline (T0)	T12	Change	*n*	Baseline (T0)	T12	Change
IL-1β (pg/ml)	17	63.84 ± 11.40 (39.6; 88.0)	86.68 ± 8.81 (67.9; 105.3)	22.83 ± 11.36 (1.2; 46.9)	13	94.61 ± 14.3 (63.3; 125.8)	94.16 ± 7.77 (77.2; 111.1)	−0.45 ± 16.71 (−36.8; 35.9)

T0: baseline. 2 hours: 2 hours after the first dose of citicoline at T0. T4: 4 weeks. T12: 12 weeks. Scores: mean ± SEM (CI 95%).

TABLE 6. Effect of citicoline on blood histamine concentrations in Alzheimer's disease patients.

		Placebo				Citicoline		
Parameter	*n*	Baseline (T0)	2 hours	Change	*n*	Baseline (T0)	2 hours	Change
HA (ng/ml)	17	61.33 ± 14.83 (29.8; 92.7)	57.50 ± 12.25 (31.5; 50.5)	−3.82 ± 20.66 (−12.2; 4.6)	13	92.35 ± 20.66 (47.7; 137.8)	92.35 ± 21.82 (44.7; 139.9)	−0.47 ± 3.52 (−8.5; 7.2)
Parameter	*n*	Baseline (T0)	T4	Change	*n*	Baseline (T0)	T4	Change
HA (ng/ml)	17	61.33 ± 14.83 (29.8; 92.7)	54.11 ± 12.77 (27.0; 81.1)	−7.22 ± 4.30 (−12.2; 4.6)	13	92.82 ± 20.66 (47.7; 137.8)	98.65 ± 24.82 (44.5; 152.7)	5.82 ± 6.75 (−8.8; 20.5)
Parameter	*n*	Baseline (T0)	T12	Change	*n*	Baseline (T0)	T12	Change
HA (ng/ml)	17	61.33 ± 14.83 (29.8; 92.7)	65.98 ± 16.19 (31.6; 100.3)	4.64 ± 4.59 (−5.1; 14.3)	13	92.82 ± 20.66 (47.7; 137.8)	96.96 ± 21.00 (51.2; 142.7)	4.13 ± 3.23 (−2.9; 11.1)
Parameter	*n*	Baseline (T0)	T13	Change	*n*	Baseline (T0)	T13	Change
HA (ng/ml)	17	61.33 ± 14.83 (29.8; 92.7)	54.21 ± 9.26 (34.5; 73.8)	−7.11 ± 11.14 (−30.7; 16.5)	13	92.82 ± 20.66 (47.7; 137.8)	80.58 ± 16.80 (43.9; 117.2)	−12.24 ± 5.80 (−24.8; 0.39)

T0: Baseline. 2 hours: 2 hours after citicoline intake in T0. T4, T12 and T13: 4, 12 and 13 weeks. Scores: mean ± SEM (CI 95%).

the cholinergic impairment is less. This finding might support both a noncholinergic-mediated effect of citicoline or the contrary. Therefore, although we do not know the cause of the genotype-response association, it is clear that Alzheimer's disease patients with the APOE4 are the best responders to citicoline.

As compared to the placebo, citicoline increased all the brain blood flow velocities when data of the two middle cerebral arteries were analyzed together, as well as diastolic velocity in the left middle cerebral artery. This positive effect of citicoline on brain hemodynamics is consistent with results of previous studies (27) showing that this compound improves perfusion deficits observed in Alzheimer's disease patients (38, 39). As in the case of cognitive performance, changes in hemodynamic parameters were more pronounced in the placebo group (worsening) than in the citicoline group (improvement), which might also be in relation with the previous intake of this medication. It is important to note that the biggest effect of citicoline on cerebral perfusion was observed in the left hemisphere, where blood flow velocities were lower.

Treatment with citicoline tended to reduce slow δ activity, potentiated α frequencies in the occipital region and increased relative θ power in the left hemisphere in patients with Alzheimer's disease. Brain bioelectrical activity of the α type increased in a nonsignificant manner in patients treated with citicoline and decreased in patients receiving placebo. Between group differences in relative α power were near to statistically significant values in occipital electrodes. This effect is similar to that previously reported in patients with late-onset Alzheimer's disease (27), and was accompanied by an opposite trend in β activity.

Although differences between groups were not significant, the decrease of δ activity was larger in patients treated with citicoline than in those receiving placebo, especially in frontal, right frontoparietal and left temporal electrodes. A significant reduction of δ frequencies was observed in the left anterior temporal electrode (T3) after treatment with citicoline. The decrease in δ activity was accompanied by a significant increase in relative θ power in subjects receiving citicoline, suggesting a partial acceleration (trend to normalization) of the EEG pattern in these patients. In several studies we have found that α frequencies decrease and δ activity increases progressively with severity of the dementia, with changes in relative θ power being less consistent (38). Regarding θ activity, differences between groups reached significant values in electrodes of the left side, which is in

concordance with the relative improvement in cerebral blood flow observed in the same hemisphere of patients treated with citicoline. These results are also in agreement with previous data showing that cognitive performance values correlated with the levels of δ (negatively) and α activity (positively) in left electrodes (27).

Circulating histamine and IL-1β levels showed no significant changes in either the placebo group or in patients treated with citicoline. However, as in previous studies (26, 27), we observed a decrease in the concentrations of IL-1β after 4 weeks of treatment with citicoline. Previously, we reported a significant reduction in serum IL-1β content induced by citicoline in early-onset Alzheimer's disease patients, but not in late-onset patients (26, 27). This kind of differential analysis based on the disease onset was not performed in the present study due to the small number of early-onset Alzheimer's disease patients included in the citicoline-treated group. As a general trend, IL-1β concentrations decreased in patients receiving citicoline and increased in the placebo group. On the other hand, modifications in blood histamine levels were not observed after citicoline treatment in this study, which is in contrast with the decrease in histamine content observed in previous investigations (27, 43). However, since this reduction in histamine levels induced by citicoline reached statistical significance only in early-onset Alzheimer's disease patients (27, 43), it is not possible to establish a suitable comparison with the present results.

According to previous investigations, in patients with Alzheimer's disease the following was observed: increased circulating histamine and IL-1β levels (28), mainly in early-onset cases; the greatest cognitive impairment in subjects with the highest concentrations of histamine and IL-1β (28); a progressive increase in histamine content with increasing disease severity (44); and correlations of these neuroinmune factors (histamine and IL-1β) with brain hemodynamic parameters (44, 45). It is interesting to note that baseline values of both histamine and IL-1β were higher in patients treated with citicoline than those in the placebo group. These differences make it difficult to interpret the changes induced by citicoline, as compared to placebo, in the levels of such biological parameters.

Finally, safety results indicate that citicoline is a safe drug and has a good pharmacological profile for the treatment of Alzheimer's disease patients because of the lack of side effects. As adverse events reported in the two treatment groups were similar, and were in fact even more frequent in the placebo group, they cannot be attributed to the treatment. Administration of citicoline did not alter arterial blood pressure, heart rate, body temperature or body weight values. Abnormalities in biological parameters related to drug intake were not observed.

In summary, our data on the effects of the treatment with citicoline in Alzheimer's disease patients indicate that this compound i) improves ADAS scores, especially in subjects with APOE4 or with APOE4 and GDS < 5, and performance in the ADAS-cog to a lesser extent; ii) potentiates cerebral perfusion, avoiding the decrease in brain blood flow velocities observed in the placebo group; iii) increases relative α and θ activity power and reduces slow δ frequencies, which indicates a positive (accelerating) effect on the brain bioelectrical activity pattern; iv) tends to reduce plasma IL-1β values, particularly after 4 weeks of treatment; and v) is well-tolerated by the patients because it does not induce adverse events nor changes in the biological parameters evaluated. According to these results, citicoline seems to be effective in treating Alzheimer's disease patients, and shows higher efficacy in APOE4 bearers with mild dementia (GDS < 5).

ACKNOWLEDGEMENTS

These experiments were conducted with grants from Ferrer International and the EuroEspes Foundation (Spain). We would like to thank J. Caamaño, B. Novo and S. Rodríguez for their technical assistance.

REFERENCES

1. Cacabelos, R., Nordberg, A., Caamaño, J. et al. *Molecular strategies for the first generations of antidementia drugs (I). Tacrine and related compounds.* Drugs Today 1994, 30: 295-337.
2. George, T.P., Cook, H.W., Byers, D.M., Palmer, F.B., Spence, M.W. *Channeling of intermediates in CDP-choline pathway of phosphatidylcholine biosynthesis in cultured glioma cells is dependent on intracellular Ca^{2+}.* J Biol Chem 1991, 266: 12419-23.
3. Trovarelli, G., Palmerini, C.A., Floridi, A., Piccini, G.L., Porcellati, G. *The influence of cytidine on the endogenous pool of CDP-choline, CDP-ethanolamine, and CAMP of the rat brain.* Neurochem Res 1984, 9: 73-9.
4. Wutman, R.J., Blusztajn, J.K., Ulus, I.H. et al. *Choline metabolism in cholinergic neurons: Implications for the pathogenesis of neurodegenerative diseases.* Adv Neurol 1990, 51: 117-25.
5. Agut, J., Font, E., Saladrich, J.M., Sacristán, A., Ortiz, J.A. *Acción farmacológica de la CDP-colina oral en un modelo de discinesia tardía en rata.* Med Clin 1986, 87 (Suppl. 1): 14-8.
6. Gaileffi, P., De Rosa, M., Cotticelli, M.G., Morana, A., Vaccaro, R., Zappia, V. *Biochemical rationale for the use of CDP-choline in traumatic brain injury: Pharmacokinetics of the orally administered drug.* J Neurol Sci 1991, 103: 19-25.
7. Giménez, R., Raïch, J., Aguilar, J. *Changes in brain striatum dopamine and acetylcholine receptors induced by chronic CDP-choline treatment in aging mice.* Br J Pharmacol 1991, 104: 575-8.
8. Martinet, M., Fonlupt, P., Pacheco, H. *Effects of cytidine-5' diphosphocholine on norepinephrine, dopamine and serotonin synthesis in various regions of the rat brain.* Arch Int Pharmacodyn, 1979, 239: 52-61.
9. Álvarez, X.A., Sampedro, C., Cacabelos, R. *Antiapoptotic effects of CDP-choline in a combined (Abeta4/hypoperfusion) animal model of hippocampal neurodegeneration.* Ann Psychiatr 1999; 7: 147-156.
10. Dorman, R.V., Dabrowiecki, Z., Horrocks, L.A. *Effects of CDP-choline and CDP-ethanolamine on the alterations in rat brain lipid metabolism induced by global ischemia.* J Neurochem 1983, 40: 276-9.
11. Kakihana, M., Fukuda, N., Suno, M., Nagaoka, A. *Effects of CDP-choline on neurologic deficits and cerebral glucose metabolism in a rat model of cerebral ischemia.* Stroke 1988, 19: 217-22.

12. Masi, I., Giani, E., Galli, C. *Effects of CDP-choline on platelet aggregation and the antiaggregatory activity of arterial wall in the rat.* Pharmacol Res Commun 1986, 18: 273-81.
13. Arrigoni, E., Averet, N., Cohadon, F. *Effects of CDP-choline on phospholipase A2 and cholinephosphotransferase activities following a cryogenic brain injury in the rabbit.* Biochem Pharmacol 1987, 36: 3697-700.
14. Cervós-Navarro, J., Lafuente, J.V. *Effect of cytidine diphosphate choline on ultraviolet-induced brain edema.* Adv Neurol 1990, 52: 421-9.
15. Álvarez, X.A., Fernández-Novoa, L., Perea, J.E., Daniele, D., Cacabelos, R. *Citicoline reverses learning impairment induced by β-amyloid injections into the rat hippocampus.* 5th Intl Geneva/ Springfield Symp Adv Alzheimer's Ther 1998, 134.
16. Miguel-Hidalgo, J.J., Álvarez, X.A., Lagares, R., Franco, A., Fernández-Novoa, L., Cacabelos, R. *Brain neurotoxic lesions in rats: Study of the neuroprotective effects of CDP-choline.* Eur Neuropsychopharmacol 1996, 6 (Suppl. 3): 193-4.
17. Miguel-Hidalgo, J.J, Álvarez, X.A., Cacabelos, R. *Neuroprotection by CDP-choline in a model of hippocampal degeneration after deposits of fragment 1-40 of β-amyloid.* Biol Psychiatry 1997, 42: 95.
18. Álvarez, X.A., Vecino, B., Perea, J.E., Daniele, D., Cacabelos, R. *CDP-choline antagonizes bromazepam-induced amnesia in rats.* Human Psychopharmacol 1997, 12: 547-56.
19. Drago, F., Mauceri, F., Nardo, L., Valerio, C., Genazzani, A.A., Grassi, M. *Effects of cytidine-diphosphocholine on acetylcholine-mediated behaviors in the rat.* Brain Res Bull 1993, 31: 485-9.
20. Petkov, V.D., Mosharrof, A.H., Kehayov, R., Petkov, V.V., Konstantinova, E., Getova, D. *Effect of CDP-choline on learning and memory processes in rodents.* Methods Find Exp Clin Pharmacol 1991, 14: 593-605.
21. Suryani, L.K., Adnjana, T.A.K., Jensen, G.D. *Citicoline treatment of memory deficits in elderly people.* Int J Geriatr Psychiat 1988, 3: 235-6.
22. Spiers, P.A., Myers, D., Hochanadel, G.S., Lieberman, H.R., Wurtman, R.J. *Citicoline improves verbal memory in aging.* Arch Neurol 1996, 53: 441-8.
23. Álvarez, X.A., Laredo, M., Corzo, D. et al. *Citicoline improves memory in elderly subjects.* Methods Find Exp Clin Pharmacol 1997, 19: 201-10.
24. Tazaki, Y., Sakai, F., Otomo, E., Kutsuzawa, T., Kameyama, M., Omae, T., Fujishima, M., Sakuma, A. *Treatment of acute cerebral infarction with a choline precursor in a multicenter double-blind placebo-controlled study.* Stroke 1988, 19: 211-6.
25. Chandra, B. *Treatment of multi-infarct dementia with citicholine.* J Stroke Cerebrovasc Dis 1992, 2: 232-3.
26. Cacabelos, R., Alvarez, X.A., Franco-Maside, A., Fernández-Novoa, L., Caamaño, J. *Effect of CDP-choline on cognition and immune function in Alzheimers disease and multi-infarct dementia.* Ann NY Acad Sci 1993, 695: 321-3.
27. Cacabelos, R., Caamaño, J., Gómez, M.J., Fernández-Novoa, L., Franco-Maside A., Álvarez, X.A. *Therapeutic effects of CDP-Choline in Alzheimer's disease. Cognition, brain mapping, cerebrovascular hemodynamics, and immune factors.* Ann NY Acad Sci 1996, 777: 399-403.
28. Álvarez, X.A., Franco, A., Fernández-Novoa, L., Cacabelos, R. *Blood levels of histamine, IL-1β and TNF-α in patients with mild to moderate Alzheimer disease.* Mol Chem Neuropathol 1996, 29: 237-52.
29. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. 10th Revision, ICD-10. WHO, Geneva 1992.
30. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Fourth Edition (DSM-IV). APA, Washington, 1994.
31. McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M. *Clinical diagnosis of Alzheimer's disease: Report of NINCDS-ADRDA Work Group.* Neurology 1984, 34: 939-44.
32. Folstein, M.F., Folstein, S.E., McHug, P.R. *Mini-mental state: A practical method for grading the cognitive state of patients for the clinician.* J Psychiat Res 1975, 12: 189-98.
33. Reisberg, B., Ferris, S.H., De Leon, M.J., Crook, T. *The global deterioration scale for assessment of primary degenerative dementia.* Am J Psychiat 1982, 139: 1136-9.
34. Hamilton, M.A. *A rating scale for depression.* J Neurol Neurosurg Psychiat 1960, 23: 56-62.
35. Rosen, W.G., Mohs, R.C., Davis, K.L. *A new rating scale for Alzheimer's disease.* Am J Psychiatry 1984, 141: 1356-64.
36. Knopman, D.S., Knapp, M.J., Gracon, S.I., Davis, C.S. *The clinician interview-based impression (CIBI): A clinician's global change rating scale in Alzheimer's disease.* Neurology 1994, 44: 2315-21.
37. Vinagre, D., Franco-Maside, A, Gómez, M.J., Caamaño, J., Cacabelos, R. *Brain electrical activity mapping in cerebrovascular disorders and senile dementia.* Drugs Today 1994, 30: 275-81.
38. Cacabelos, R., Caamaño, J., Vinagre, D., Lao, J.I., Beyer, K., Álvarez, X.A. *Brain mapping and transcranial Doppler ultrasonography in Alzheimer Drug monitoring.* In: Alzheimer Disease: From Molecular Biology to Therapy. Becker, R., Giacobini, E. (Eds.). Birkhauser: Boston 1996, 469-73.
39. Caamaño, J., Gómez, M.J., Vinagre, D., Franco-Maside, A., Cacabelos, R. *Transcranial Doppler ultrasonography in senile dementia.* Drugs Today 1994, 30: 283-93.
40. Beyer, K., Lao, J.I., Álvarez, X.A., Cacabelos, R. *Differential implications of APOE E4 in Alzheimer's disease and vascular dementia in the Spanish population.* Alzheimer's Res 1996; 2: 215-20.
41. Davis, K.L., Thal, L.J., Gamzu, E.R. et al. and the Tacrine Collaborative Group. *A double-blind, placebo-controlled multicenter study of tacrine for Alzheimers disease.* N Engl J Med 1992, 327: 1253-59.
42. Richard, F., Helbecque, N., Guez, D., Levy, R., Amouyel, Ph. *APOE genotyping and response to drug treatment in Alzheimer's disease.* The Lancet 1997, 349: 539.
43. Fernández-Novoa, L., Álvarez, X.A., Franco, A., Cacabelos, R. *CDP-choline-induced blood histamine changes in Alzheimer's disease.* Meth Find Exp Clin Pharmacol 1994, 16: 279-84.
44. Fernández-Novoa, L., Álvarez, X.A., Zas, R., Corzo, L., Cacabelos, R. *Global deterioration scale-related brain haemodynamics and histamine levels in Alzheimer disease and vascular dementia.* Ann NY Acad Sci 1997, 826: 396-400.
45. Álvarez, X.A., Fernández-Novoa, L., Caamaño, J. et al. *Cerebrovascular changes associated with interleukin-1beta (IL-1beta) and histamine levels in Alzheimer's disease.* Ann NY Acad Sci 1997, 826: 375-8.

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