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The ASCOMALVA (Association between the Cholinesterase Inhibitor Donepezil and the Cholinergic Precursor Choline Alphoscerate in Alzheimer's Disease) Trial: Interim Results after Two Years of Treatment

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Abstract. Cholinesterase inhibitors (ChE-Is) are used for symptomatic treatment of mild-to-moderate Alzheimer's disease (AD), but long-term effects of these compounds are mild and not always obvious. Preclinical studies have shown that combination of ChE-Is and the cholinergic precursor choline alphoscerate increases brain acetylcholine levels more effectively than single compounds alone. ASCOMALVA (Effect of association between a ChE-I and choline alphoscerate on cognitive deficits in AD associated with cerebrovascular injury) is a double-blind trial investigating if the ChE-I donepezil and choline alphoscerate in combination are more effective that donepezil alone. The trial has recruited AD patients suffering from ischemic brain damage documented by neuroimaging and has completed 2 years of observation in 113 patients of the 210 planned. Patients were randomly allotted to an active treatment group (donepezil + choline alphoscerate) or to a reference group (donepezil + placebo). Cognitive functions were assessed by the Mini-Mental State Evaluation and Alzheimer's Disease Assessment Scale Cognitive subscale. Daily activity was evaluated by the basic and instrumental activities of daily living tests. Behavioral symptoms were assessed by the Neuropsychiatric Inventory. Over the 24-month observation period, patients of the reference group showed a moderate time-dependent worsening in all the parameters investigated. Treatment with donepezil plus choline alphoscerate significantly slowed changes of the different items analyzed. These findings suggest that the combination of choline alphoscerate with a ChE-I may prolong/increase the effectiveness of cholinergic therapies in AD with concomitant ischemic cerebrovascular injury.

Keywords: Alzheimer's disease, association, cerebrovascular injury, choline alphoscerate, donepezil

INTRODUCTION

The cholinergic system plays a relevant role in learning and memory. The observation of a loss of the acetylcholine biosynthetic enzyme choline acetyltransferase in the cerebral cortex of Alzheimer's

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disease (AD) has allowed the development of the so-called "cholinergic hypothesis" of adult-onset cognitive dysfunction. Strategies for enhancing cholinergic neurotransmission were therefore proposed to counter cognitive dysfunction typical of AD [1–5]. The first therapies introduced into the clinical practice for symptomatologic treatment of AD were cholinergic precursors, followed by inhibitors of the acetylcholine catabolic enzymes acetylcholinesterase (AChE) and cholinesterases (ChE) [6]. Controlled clinical trials with choline or the choline-containing phospholipid phosphatidylcholine (lecithin) failed to show relevant effects [2]. These negative results [2, 7], although should not be extended to all cholinergic precursors [8, 9], were probably the cause of the discontinuation of the precursor loading strategy for treating AD. ChE inhibitors (ChE-Is) increase acetylcholine availability at the synaptic cleft, by slowing down its enzymatic degradation. These compounds are among the few drugs currently approved for the symptomatic treatment of AD. A large number of clinical trials have investigated the activity of these compounds. Retrospective analysis of the main studies with ChE-Is suggests that there are small beneficial long-term effects of these compounds on the cognitive, functional, and behavioral symptoms of AD. These effects are not always apparent in clinical practice [10–12].

Combination of a cholinergic precursor with a ChE-I probably could enhance cholinergic neurotransmission, because the precursor makes available more substrate for acetylcholine synthesis, whose degradation is slowed down by the ChE-I. Based on this hypothesis, preclinical studies have shown that association of the cholinergic precursor choline alphoscerate (alpha-glyceryl-phosphorylcholine) with a ChE-I significantly enhance cholinergic neurotransmission [13], and exert a more remarkable neuroprotective effect than single compounds alone [9, 14]. Relevant cholinergic deficits are observed when dementia is associated with cerebrovascular impairment [5, 15]. The possible explanation is that cholinergic neurons of the basal forebrain, which are involved in cognitive activities such learning and memory and supply cerebrovascular tree, are particularly sensitive to ischemic damage [16].

A clinical study (Association between the cholinesterase inhibitor donepezil and the cholinergic precursor choline alphoscerate in Alzheimer's disease, ASCOMALVA) was designed to assess if the association between cholinergic precursor and ChE-I, effective in preclinical studies [13, 14], may represent a therapeutic option for enhancing the effect of cholinergic therapies in AD patients with concomitant ischemic cerebrovascular disease. An interim analysis of the first data of the ASCOMALVA trial after one year of treatment was published [17]. This paper presents the interim results of the trial at the end of the second year of treatment.

METHODS

ASCOMALVA is a multicenter, randomized, placebo-controlled, and double-blind study clinical study of spontaneous generation. It has recruited all the planned subjects ($n = 210$, 132 females and 78 males), aged between 59 and 93 years (average 77 years) and has completed 2 years of observation in 113 patients (Table 1). Centers involved were the Alzheimer's Unit of Cardarelli Hospital in Naples, Italy (Unità Valutativa Alzheimer e Malattie Involutive Cerebrali, Azienda Ospedaliera di Rilievo Nazionale A. Cardarelli, Napoli) and Division of Neurology of Poma General Hospital in Mantua, Italy (Unità Complessa di Neurologia, Azienda Ospedaliera C. Poma, Mantova). Supervision, organization, and information technology support and statistics were provided by Centro Ricerche Cliniche, Telemedicina e Telefarmacia of Camerino University (Camerino, Italy). Patients' clinical data were downloaded into a computerized file on the website developed by Camerino University for ASCOMALVA [17]. Also under responsibility of the Clinical Research Center of Camerino University were randomization of patients and assignment to the two treatment groups (donepezil + placebo or donepezil + choline alphoscerate) [17].

Initially, the protocol considered to treat patients with donepezil + choline alphoscerate (treatment group) or donepezil plus placebo (control group) for

Table 1
Demographic data of patients recruited for the ASCOMALVA trial

Gender (M/F)	♂ 78 (37%) / ♀ 123 (63%)		
Age		77 ± 7	
	Min 59	Max 93	Median 79
Scolarity		7 ± 4	
	Min 2	Max 17	Median 5
MMSE at baseline		20 ± 3	
	Min 14	Max 24	Median 20
	Donepezil		Donepezil + choline alphoscerate
Gender (M/F)	♂ 22 (40%) / ♀ 34 (60%)		♂ 24 (42%) / ♀ 33 (58%)
Age	78 ± 5		76 ± 8
Scolarity	7 ± 3		8 ± 5
MMSE at baseline	20 ± 3		20 ± 3

24 months starting from enrolment, with interim evaluations planned at 3, 6, 9, 12, and 18 months. A recent amendment of the protocol prolonged the study for additional 2 years. Hence, at the conclusion, ASCOMALVA will include observation of patients for 4 years of treatment.

Maintaining the double-blind study, the protocol provides that the supervising Center may assess via WEB the advancement of the parameters analyzed at the completion of the 3, 6, 9, 12, 18, and 24 months of treatment. The mid-term evaluation, without the opening of the blindness, was possible because all data of the study, excluding the identity of patients, were available in the WEB platform operated by the coordinating Center. Only the coordinator was aware of the type of treatment (active or reference) assigned to individual patients.

The trial recruited patients suffering from AD with concomitant cerebrovascular damage. These patients represent a population with a relevant cholinergic hypofunction [18, 19] that theoretically can obtain benefit from a sustained cholinergic load. Diagnosis of AD was established according to NICDS ADRDA criteria, and vascular damage was evaluated using the New Rating Scale for Age-Related White Matter Changes (ARWMC), based on cerebral ischemic injury evaluation with computed tomography and/or brain MRI. Patients recruited should have a score ≥ 2 at the ARWMC scale and present at least two of the following vascular risk factors: hypertension, diabetes, obesity, ischemic heart disease, dyslipidemia, hyperhomocysteinemia, smoking, previous cerebrovascular events and family history of cardio-cerebrovascular diseases. Inclusion and exclusion criteria are detailed in a previous study of our group [17].

Eligible patients, after signing the informed consent, were randomly assigned to one of the treatment groups: 1) Active treatment: ChE-I (donepezil 10 mg/day) + precursor cholinergic (choline alphoscerate 1,200 mg/day); 2) Reference treatment of ChE-I (donepezil 10 mg/day) + placebo.

Among the three ChE-Is available on the pharmaceutical market in Italy (donepezil, rivastigmine, and galantamine), donepezil was chosen as it is the most largely prescribed and presents the advantage of once-a-day administration. Two years of treatment were reached by 113 patients (67 female and 46 male) and they underwent follow-up controls at 3, 6, 9, 12, and 18 months. During each follow-up visit, patients were examined using the tests listed below:

- Mini-Mental State Examination (MMSE) and Alzheimer's Disease Assessment Scale Cognitive subscale (ADAS-cog) to assess global cognitive status.
- Basic Activities of Daily Living (BADL) and Instrumental Activities of Daily Living (IADL) for the evaluation of basic and instrumental activities of daily living.
- Neuropsychiatric Inventory frequency x severity (NPI-F) and distress of the caregiver (NPI-D) for assessing the severity of neuropsychiatric symptoms and caregiver distress.

Statistical analysis of possible differences between the scores of different tests in the two study groups (donepezil + choline alphoscerate versus donepezil + placebo) was made by the analysis of variance (ANOVA). Significance of differences between the two groups was assessed by the two-tailed Student *t*-test.

RESULTS

Twelve patients allotted to the reference treatment (11.4%) and 17 patients (16.2%) allotted to the association treatment withdrew from the study. Tolerability to treatment was similar in the two patient's groups. Withdrawal reasons are summarized in Table 2.

Data of cognitive assessment (MMSE and ADAS-cog) in AD patients throughout the study are summarized in Fig. 1A and B. In the control group treated with donepezil + placebo, a progressive time-

Table 2
Patient withdrawals after two years of treatment

Therapy Causes of withdrawal	No.	Donepezil + placebo	No.	Donepezil + choline alphoscerate
Total		12		17
Death	0		1	
Lack of efficiency	0		2	Worsening of the Disease
Non compliance	3	Transferred to geriatric homecare support	3	Transferred to geriatric homecare support
Lack tolerability	3	1 Hallucinations, asthenia	3	1 Hallucinations, insomnia
		2 Diarrhea, vomiting		1 Diarrhea, vomiting
				1 Cutaneous rash
Other reasons	6	5 Problems in reaching the hospital	8	3 Problems in reaching the hospital
		1 Unknown		2 Home/city change
				3 Unknown

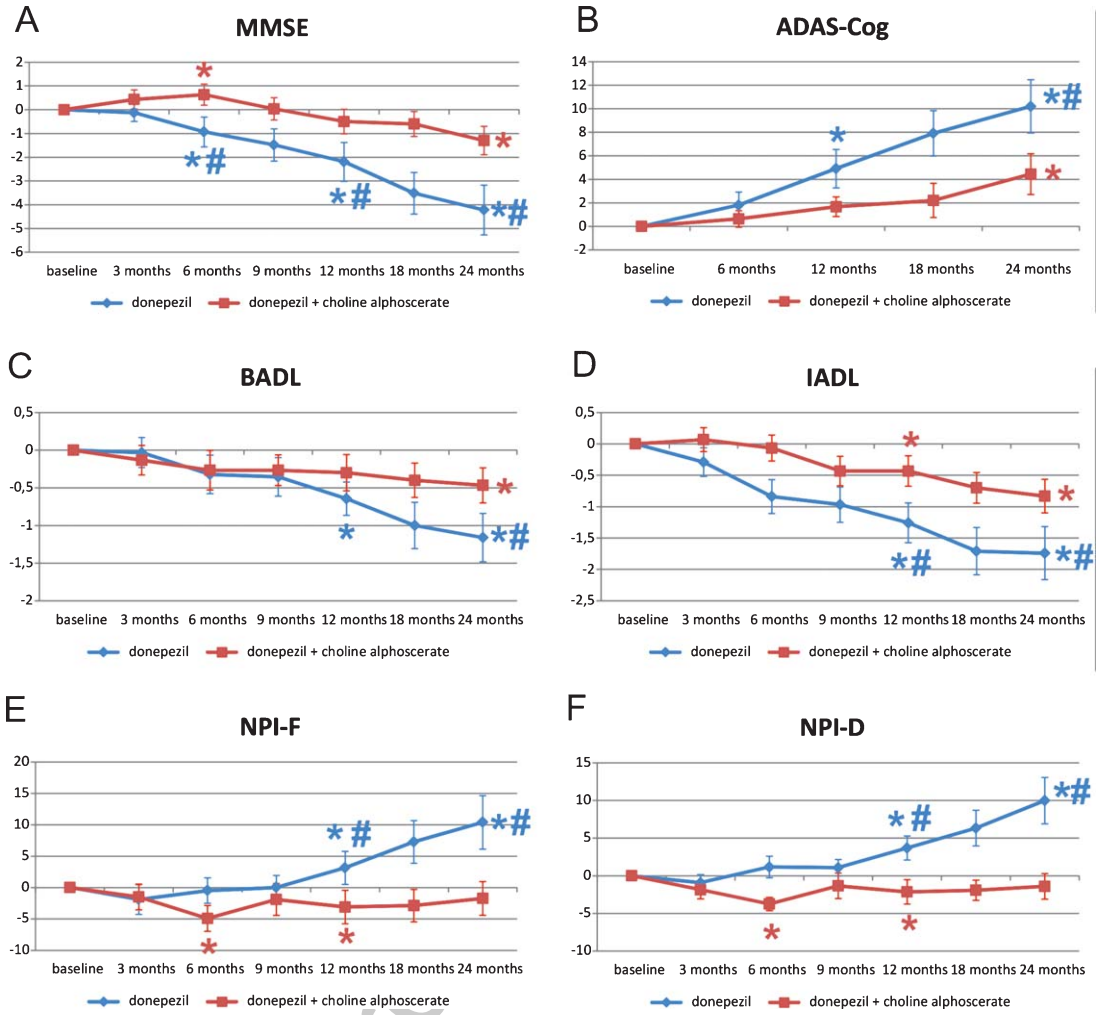

Fig. 1. Evaluation of the cognitive (MMSE, A; ADAS-cog, B), functional (BADL, C; IADL, D), and behavioral (NPI-F, E; NPI-D, F) tests during the 2 years of observation analyzed of the ASCOMALVA trial. Data are the means ± S.E.M. *$p < 0.05$ versus baseline; #$p < 0.05$ donepezil versus association therapy.

dependent worsening of MMSE and ADAS-cog scores was observed. Treatment with donepezil + choline alphoscerate (active treatment) countered this decline in cognitive tests. The effect of association on psychometric tests was statistically significant after 12 and 24 months of treatment (Fig. 1A, B).

Functional evaluation showed a decrease in BADL and IADL values in both treatment groups. BADL scores were significantly different between control group and the donepezil + choline alphoscerate groups (Fig. 1C) after two years of treatment. IADL scores were improved in active treatment patients compared to the reference group at 12 and 24 months of observation (Fig. 1D).

Data from the NPI, including analysis severity (NPI-F, Fig. 1E) and caregiver distress measures (NPI-D, Fig. 1F), at 12 months of observation and after two years of treatment, revealed a significant decrease in NPI severity and distress of caregiver scores in patients treated with donepezil + choline alphoscerate compared with those receiving treatment with donepezil alone.

Data from the above tests (psychometric, functional, and behavioral) were also analyzed according to the MMSE score at the baseline. MMSE scores were divided into the three classes listed below: a) 24-21(mild dementia); b) 20-18 (mild-moderate dementia); c) 17-15 (moderate dementia).

Analysis of data of psychometric test revealed the most remarkable improvement in cognitive functions in the mild dementia patients group either for reference and active treatment. The most marked worsening in

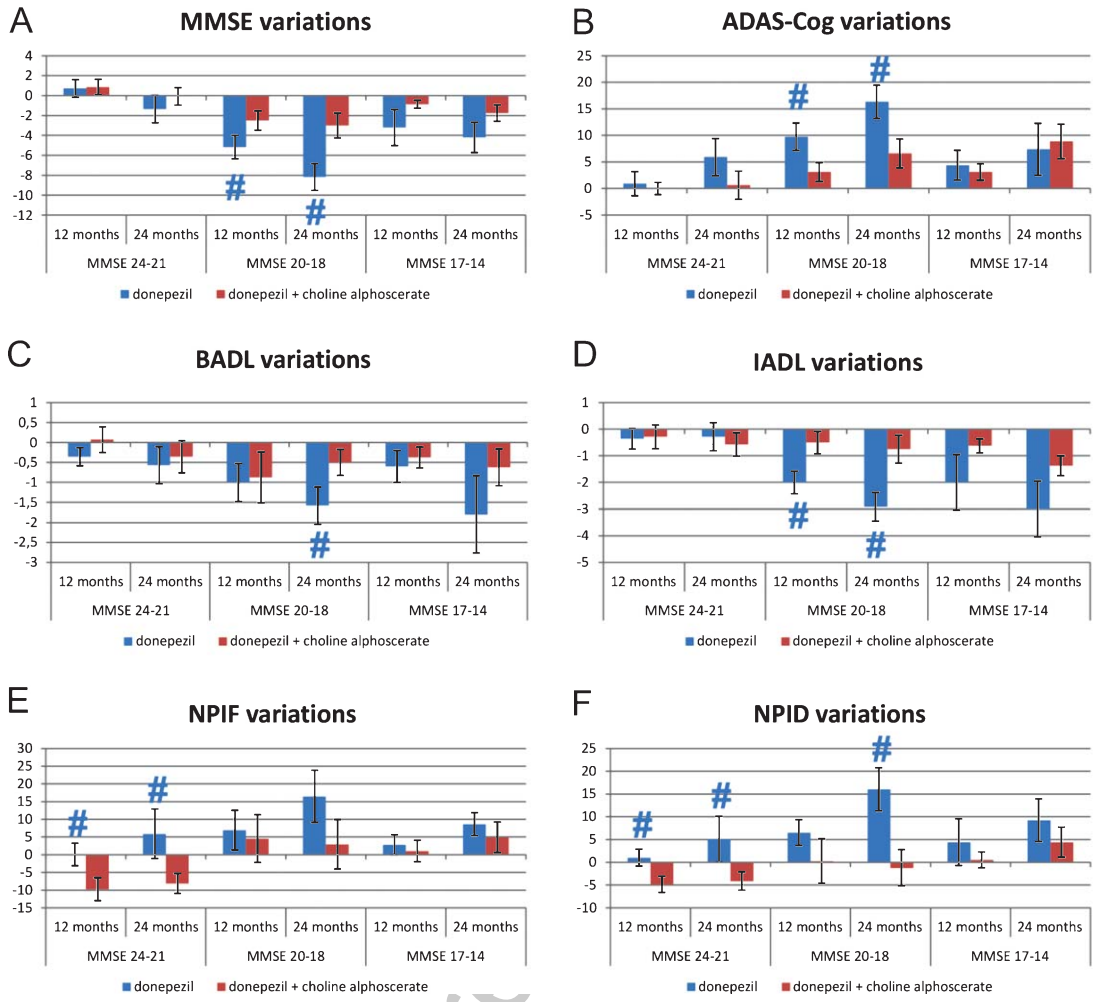

Fig. 2. Evaluation of the progression in the cognitive (MMSE, A; ADAS-cog, B), functional (BADL, C; IADL, D), and behavioral (NPI-F, E; NPI-D, F) tests after stratification by MMSE at baseline: 25>MMSE>20; 21>MMSE>17; 18>MMSE>14. Histograms summarize the variation of parameters investigated in the different groups. Data are the means ± S.E.M.; #$p < 0.05$ donepezil versus association therapy.

cognitive tests scores was observed in the group with intermediate MMSE. In this group, the combination of donepezil and choline alphoscerate was more effective versus donepezil alone in countering cognitive impairment (Fig. 2A, B).

Similar results were observed in functional tests, in which treatment with donepezil + choline alphoscerate was more effective in the groups with mild to moderate and moderate dementia at baseline (Fig. 2C, D).

Behavioral analysis and the stress of the caregiver evaluation revealed a significant improvement in patients treated with donepezil + choline alphoscerate primarily in the group with higher MMSE at baseline after two years of treatment (Fig. 2E, F).

Data of MMSE progression were interpolated by linear regression for estimating the time needed to reach a severe dementia score (MMSE <10). Regression line was calculated at least on 5–7 points (starting from the point of maximum MMSE value), and showed a mean coefficient of correlation of 90.9% (minimum 71.9%, maximum 98.7%) (Fig. 3). The results summarized in Table 3 show that association therapy increases the mean time needed to reach the severe dementia score. The most relevant differences were noticeable in the lower MMSE groups in which the estimated time values approximately doubled.

DISCUSSION

The interim results of the ASCOMALVA trial reported here, which include approximately the 50%

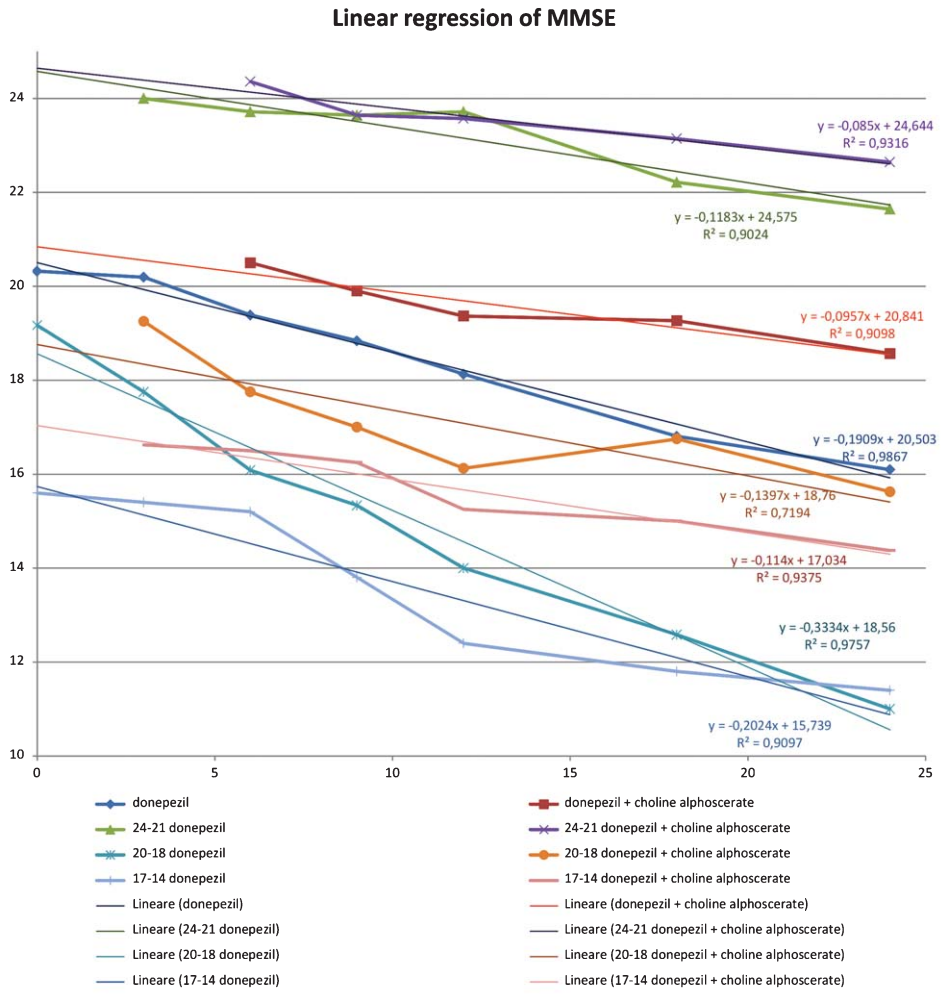

Fig. 3. Linear regression of the MMSE, data show the progression of the parameter in the two therapy groups before and after stratification by MMSE at baseline.

Table 3
Time of convergence to severe dementia (MMSE<10)

	Donepezil	Donepezil + Choline Alphoscerate
24 months	55 months	113 months
24 months MMSE 24-21	123 months	172 months
24 months MMSE 20-18	26 months	62 months
24 months MMSE 17-15	28 months	66 months

of patients planned (113 of 210) completing 2 years of treatment, indicate that association between the ChE-I donepezil and the cholinergic precursor choline alphoscerate induces cognitive and behavioral improvements better than those obtained with the ChE-I alone. These findings are consistent with and support previous interim data obtained after one year of observation [17]. After 2 years of treatment, the differences between reference (donepezil) and association (donepezil + choline alphoscerate) were more obvious suggesting that the combined therapy may slow-down the decline in the effectiveness of ChE-I therapies reported after 1–2 years of treatment [10–12].

ChE-Is represent the pharmacotherapeutic strategies more extensively used for enhancing cholinergic neurotransmission in AD patients. Some authors suggest a neuroprotective effect of this class of drugs that could slow the neurodegeneration in AD [20]. One of the main problems of ChE-I therapy is the time-dependent decrease of efficacy of treatment. Another problem is how to treat particular categories of patients (very old individuals over 85 years, or patients with bradycardia, bronchial asthma, or chronic obstructive pulmonary disease) in which ChE-I are not indicated [8]. The use of higher doses of ChE-I shows greater

efficiency, but is also associated with potentially relevant side effects, such as bradycardia, that is relatively common in patients treated with ChE-I [21, 22]. On the whole the benefits and cost/benefit ratio of AChE/ChE-I are modest and for some studies of doubtful clinical significance [23].

Cholinergic precursors were one of the first approaches proposed for treating AD, but these compounds were sparsely investigated in clinical settings, versus ChE-I and just a few molecules demonstrated some effectiveness [2]. Choline alphoscerate is probably the cholinergic precursor which has shown the greater clinical activity and a good tolerability in patients with mild-to-moderate AD and vascular dementia [8, 9]. The compound crosses the blood-brain barrier and probably acts as a donor of metabolically active choline to the brain. It has been already demonstrated that it can have a neuroprotective effect in experimental animals with vascular brain injury [9].

Cholinergic structures of the basal forebrain, which are involved in cognitive activities, are particularly susceptible to ischemia [16]. This may explain the cholinergic deficits observed in dementia in which neurodegenerative and vascular components are associated [5, 15]. In view of this, patients recruited for ASCOMALVA were suffering from AD associated with vascular damage. They therefore represent a patient population with a marked cholinergic hypofunction [18, 19], that could benefit from the cholinergic challenge provided by the association of high doses of choline alphoscerate plus 10 mg/day donepezil. The association of cholinergic precursors plus ChE-Is such as tacrine or physostigmine was already tried, but showed no evident effect, versus the treatment with ChE-Is alone [7]. Possible reasons of this lack of effect were the short duration of these trials or the use of inappropriate precursors or inhibitors.

In the ASCOMALVA trial, our interim data showed that after 2 years of treatment cholinergic association induced clinical effects more pronounced than the ChE-I alone and slowed the progressive decline in therapeutic responsiveness, which is common with long term administration of ChE-I. Activity of the association was more marked in patients with mild dementia, but it was also effective in patients with greater cognitive impairment, in which the ChE-I monotherapy was almost ineffective.

CONCLUSIONS

Pathological, epidemiological, and retrospective studies have demonstrated that AD patients with associated vascular injury represent the highest percentage of late-onset dementia population [3, 15, 24]. The consequences of the association between neurodegenerative and vascular phenomena in the pathophysiology and clinical course of AD are still under discussion. A strong relationship between vascular impairment, such as cerebral micro-bleeds, and dementia in AD was suggested [25–27]. AD patients with associated cerebrovascular injury make the largest group of elderly demented, and probably, those in which the progression of disease is more aggressive [18].

Lacking new or more effective therapies for AD, a reasonable approach could be represented by the combination/optimization of available treatments. The interim results reported here of the ASCOMALVA trial suggest that the cholinergic association used could represent a therapeutic option to be considered in the treatment of AD associated with cerebrovascular damage. Further data will follow with the advancement of the study.

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