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The ASCOMALVA trial: Association between the cholinesterase inhibitor donepezil and the cholinergic precursor choline alphoscerate in Alzheimer's disease with cerebrovascular injury: Interim results

Francesco Amenta [a,*], Anna Carotenuto [a], Angiola Maria Fasanaro [b], Raffaele Rea [a], Enea Traini [a]

[a] Centro Ricerche Cliniche, Scienze del Farmaco e dei Prodotti della Salute, Università di Camerino, Camerino, Italy
[b] Unità Valutativa Alzheimer e Malattie Involutive Cerebrali, Azienda Ospedaliera di Rilievo Nazionale A. Cardarelli, Napoli, Italy

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ABSTRACT

Background: Cholinesterase inhibitors (ChE-Is) are among the drugs more largely used for the treatment of mild-to-moderate symptoms of Alzheimer's disease (AD), but beneficial long-term effects of these compounds on the cognitive, functional, and behavioural symptoms of the disease are small and not always apparent in practice. Preclinical investigations have suggested that association between ChE-Is and the cholinergic precursor choline alphoscerate enhances cholinergic neurotransmission more effectively than single compounds alone. The ongoing clinical trial on the "Effect of association between a ChE-I and choline alphoscerate on cognitive deficits in Alzheimer's disease associated with cerebrovascular injury" (ASCOMALVA) was designed to assess if association of the ChE-I donepezil with choline alphoscerate has a more favourable clinical profile than monotherapy with donepezil alone.
Methods: ASCOMALVA is a double-blind multicentre trial that has completed the first 12 months of observation of 91 patients of the 210 planned. Patients were aged between 56 and 91 years (mean 75 ± 10 years) and were included in the protocol with a MMSE score between 15 and 24. Patients with AD diagnosed according to the DSM IV criteria suffer from ischemic brain damage documented by neuroimaging (MRI and CT scan), with a score ≥ 2 in at least one subfield of the New Rating Scale for Age-Related White Matter Changes (ARWMC). Patients were randomly allotted to an active treatment group (donepezil + choline alphoscerate) or to a reference treatment group (donepezil + placebo) and were examined after 3, 6, 9 and 12 months of treatment.
Results: Cognitive functions, patient's daily activities and behavioural symptoms were assessed by the Mini-Mental State Evaluation (MMSE), Alzheimer's Disease Assessment Scale Cognitive subscale (ADAS-cog), Basic Activities of Daily Living (BADL), Instrumental Activities of Daily Living (IADL) and Neuropsychiatric Inventory (NPI), of severity and of caregiver distress measures (NPI-F and NPI-D). Patients of the reference group (donepezil + placebo) showed along the course of the 12 months of observation, a slight time-dependent worsening of MMSE, ADAS-cog, IADL and NPI-D scores and no changes in the BADL and NPI-F scores. Donepezil plus choline alphoscerate improved compared to donepezil alone the different items analysed except the BADL.
Conclusions: The first results of the ASCOMALVA trial suggest that association of choline alphoscerate to the standard treatment with a ChE-I may represent an option to prolong beneficial effects of cholinergic therapies in AD with concomitant ischemic cerebrovascular injury.

1. Introduction

The so-called cholinergic hypothesis of adult-onset cognitive dysfunction, based on the observation of a loss of the acetylcholine biosynthetic enzyme choline acetyltransferase in the cerebral cortex of Alzheimer's disease (AD) has allowed to identify the cholinergic system as one of the neurotransmitter pathways with a relevant role in learning and memory. Based on it cholinergic strategies to counter cognitive dysfunction typical of adult-onset dementia, including AD were also developed [1–5]. The main cholinergic therapies introduced in clinical practice included cholinergic precursors and inhibitors of the acetylcholine catabolic enzymes acetylcholinesterase (AChE) and cholinesterase (ChE) [6]. Cholinergic precursor loading therapy was the first approach tried to relieve cognitive impairment in AD, although controlled clinical trials failed to show relevant effects induced by choline or the choline-containing phospholipid phosphatidylcholine (lecithin). The reasons for the lack of effect of this precursor strategy are unclear [2], but negative results obtained with choline or phosphatidylcholine [2,7] cannot be generalized for all cholinergic

* Corresponding author at: Centro Ricerche Cliniche, Telemedicina e Telefarmacia Università di Camerino, Via Madonna delle Carceri 9, 62032 Camerino (MC), Italy. Tel.: +39 0737 403311/403326; fax: +39 0737 403325.
E-mail address: francesco.amenta@unicam.it (F. Amenta).

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precursors [for a review see 8]. ChE inhibitors (ChE-Is) increase acetylcholine availability at the synaptic cleft, by slowing down its enzymatic degradation, and are currently approved for the symptomatic treatment of AD. The activity of these compounds was investigated in numerous clinical trials, but a retrospective analysis of the main studies with ChE-I suggests that beneficial long-term effects of these compounds on the cognitive, functional, and behavioural symptoms of AD are small and not always apparent in practice [9–11].

From a theoretical point of view, association of a cholinergic precursor with a ChE-I may enhance cholinergic neurotransmission. In fact, the precursor could make available more substrate for acetylcholine synthesis, the degradation of which is slowed down by the ChE-I. In line with this hypothesis are preclinical studies showing that association of the cholinergic precursor choline alphoscerate (alpha-glyceryl-phosphorylcholine) with a ChE-I significantly enhances cholinergic neurotransmission [12], and exerts a more remarkable neuroprotective effect than single compounds alone [13].

The present clinical study was designed to assess if the cholinergic precursor/ChE-I association documented to be effective in preclinical studies [12,13] may represent a therapeutic option to prolong/increase beneficial effects of cholinergic therapies in AD patients with concomitant ischemic cerebrovascular disease.

2. Methods

ASCOMALVA is a multicentre, randomized, placebo-controlled, double-blind clinical trial. It has included, so far, 183 subjects (105 female and 78 male), aged between 56 and 91 years (average 75), of the 210 planned (Fig. 1). Centres involved were 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 informatics support and statistics were provided by Clinical Research Centre of Camerino University, Camerino, Italy. Clinical data were put into a computerized file on the net developed for ASCOMALVA. Each researcher can access the clinical files using a personal password. For increasing the personal data protection, each centre prepared a numerical list of patients. The identity of subjects was kept not in the WEB, but was taken protected by the investigating centre only. The numerical list was sent to the Clinical Research Centre of Camerino University for randomization purposes and for allotting patients to one of the two treatments planned (donepezil + placebo or donepezil + choline alphoscerate). Randomization lists were prepared in groups of 20 subjects per centre. Hence, each centre could work independently with the advantage of an easy possible combination of the results obtained. This WEB-based procedure was followed also for economical reasons, being ASCOMALVA a clinical study of spontaneous generation.

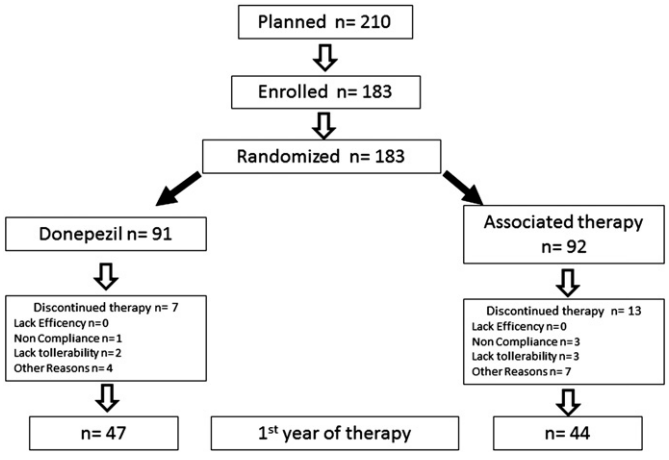

Fig. 1. Flowchart of ASCOMALVA trial and recruitment of the patients.

The study protocol includes the treatment of patients with donepezil + choline alphoscerate (treatment group) or donepezil + placebo (control group) for 24 months starting from enrolment in the study and with interim assessments at 3, 6, 9, 12, and 18 months. While maintaining the double-blind study, the protocol provides that the supervising Centre may assess via WEB the progress of the parameters analysed at the achievement of the 3, 6, 9, 12, 18 and 24 months of treatment. These interim evaluations are primarily focused on the withdrawal of active treatment of the association in case of lack of apparent results of the same. The mid-term evaluation, without the opening of the blinds, was possible because all data of the study, excluding the identity of patients, were available in a web platform operated by the coordinating Centre.

Patients were selected according to the following criteria: the diagnosis of AD associated with vascular damage was placed using NINCDS ADRDA criteria.

The criteria for inclusion in the study were:

- Age > 50 years;
- Mini-Mental State Evaluation (MMSE) [14] between 24 and 12;
- Score ≥ 2 at the New Rating Scale for Age-Related White Matter Changes (ARWMC), the rating scale of cerebral ischemic injury evaluated with computed tomography and/or brain MRI [15];
- Presence of at least two of the following vascular risk factors: hypertension, diabetes, obesity, ischemic heart disease, dyslipidemia, hyperhomocysteinemia, smoking, previous cerebrovascular events and familiar history of cardio-cerebrovascular diseases.

Exclusion criteria were:

- Decompensated heart disease;
- Chronic renal failure;
- Severe liver failure;
- Incorrect dysthyroidism;
- Developmental disorders (eg cancer);
- Conditions that could interfere with assessments of safety/efficacy;
- Diagnosis of major depression (according to DSM IV criteria).

Eligible patients, after signing the informed consent, were randomly assigned to one of the treatment groups indicated below.

- Active treatment: cholinesterase inhibitor (donepezil 10 mg/day) + precursor cholinergic (choline alphoscerate 1200 mg/day)
- Reference treatment of cholinesterase inhibitor (donepezil 10 mg/day) + placebo.

The first part of the treatment period of 12 months was completed by 91 of the patients (58 female and 33 male) and they underwent follow-up controls at 3, 6 and 9 months. During each follow-up each patient underwent a series of tests including:

- 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.

The statistical analysis of the scores of the various evaluations was conducted with the analysis of variance (ANOVA), for the identification of possible significance of differences between the two study groups (donepezil + choline alphoscerate vs. donepezil + placebo); followed by a two sided Student "t" test to evaluate the differences within the groups during treatment.

Table 1
Reasons for patients' withdrawal from the ASCOMALVA trial.

Therapy / Causes of withdrawal	No.	Donepezil + placebo	No.	Donepezil + choline alphoscerate
Lack of efficency	0		0	
Noncompliance	1	Transferred to geriatric homecare support	3	Transferred to geriatric homecare support
Lack tollerability	2	1 Hallucinations, asthenia	3	1 Hallucinations, insomnia
				1 Diarrhea, vomiting
		1 Diarrhea, vomiting		1 Cutaneous rash
Other reasons	4	2 Problems in reaching the hospital	7	2 Problems in reaching the hospital
				2 Home/city change
		2 Unknown		3 Unknown

3. Results

Treatment was discontinued by 7 patients allotted to the reference treatment (7.7%) and 13 patients (14.1%) allotted to the association treatment. Tolerability of treatment was similar in the two patients' groups. Withdrawal reasons are summarized in Table 1.

Data of cognitive assessment (MMSE and ADAS-cog) in AD patients throughout the study are summarized in Fig. 2A and B. As shown, in the control group (donepezil + placebo) a slight time-dependent worsening of MMSE and ADAS-cog scores was found. Treatment with donepezil + choline alphoscerate (active treatment) countered the decline of MMSE and ADAS-cog scores. The effect of association on psychometric tests was statistically significant after 12 months of treatment (Fig. 2A and B). BADL scores were unchanged between the control group and the donepezil + choline alphoscerate groups (Fig. 2C), whereas IADL scores were improved in active treatment patients compared to the reference group at 12 months of treatment (Fig. 2D).

Data of Neuropsychiatric Inventory (NPI), including analysis severity (NPI-F) and caregiver distress measures are shown in Fig. 3A and C. Analysis of the percentage variations in NPI-F (Fig. 3B) and NPI-D (Fig. 3D) scores at 12 months of observation revealed a significant decrease of NPI severity and distress of caregiver scores in patients treated with donepezil + choline alphoscerate compared with those receiving treatment with donepezil alone.

Data of different psychometric, functional and behavioural tests were also analysed independently according to the MMSE score at the baseline divided into the three classes listed below: a) 24–21; b) 20–18; c) 17–15. Analysis of MMSE scores revealed the best activity of the association with the lowest MMSE score at the baseline (Fig. 4). Treatment with donepezil + choline alphoscerate had a better influence on ADAS-cog scores primarily in patients with a MMSE ranging 20–18 at the baseline (Fig. 5). The association influenced positively BADL values only in the MMSE group 24–21 (Fig. 6A), IADL in the MMSE group 17–15 (Fig. 6B), whereas NPI-F and NPI-D did significantly improve in all MMSE groups, with a greater effectiveness in the MMSE group 20–18 (Fig. 6C and D).

4. Discussion

The interim results of the ASCOMALVA trial reported here, which include approximately the 50% of patients planned (91 of 210) and half of the time of the observation (1 year of the 2 years planned) indicate that association between the ChE-I donepezil and the cholinergic precursor choline alphoscerate induces cognitive and behavioural improvements superior than those obtained with the ChE-I alone. In contrast functional analysis revealed a lower sensitivity to the association of tests used (BADL and IADL). As mentioned above, ASCOMALVA is a controlled, double-blind multicentre study involving two AD Clinics (Mantua and Naples) held in collaboration with Clinical Research Centre of Camerino University. In order to guarantee the homogeneity of the trial in spite of the distance between the 2 AD Clinics (more than 500 km), the trial was developed and carried out using a sophisticated

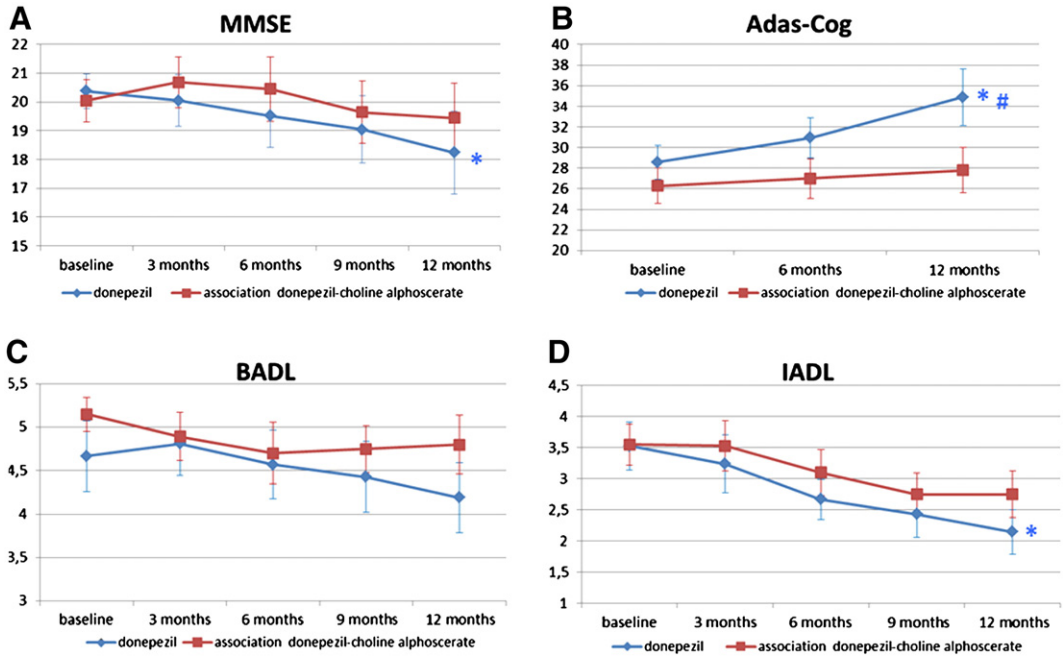

Fig. 2. Evaluation of the cognitive (MMSE, A; ADAS-cog, B) and functional (BADL, C; IADL, D) tests. Data are the means ± S.E.M. *$p<0.05$ vs. baseline; #$p<0.05$ donepezil vs. association therapy.

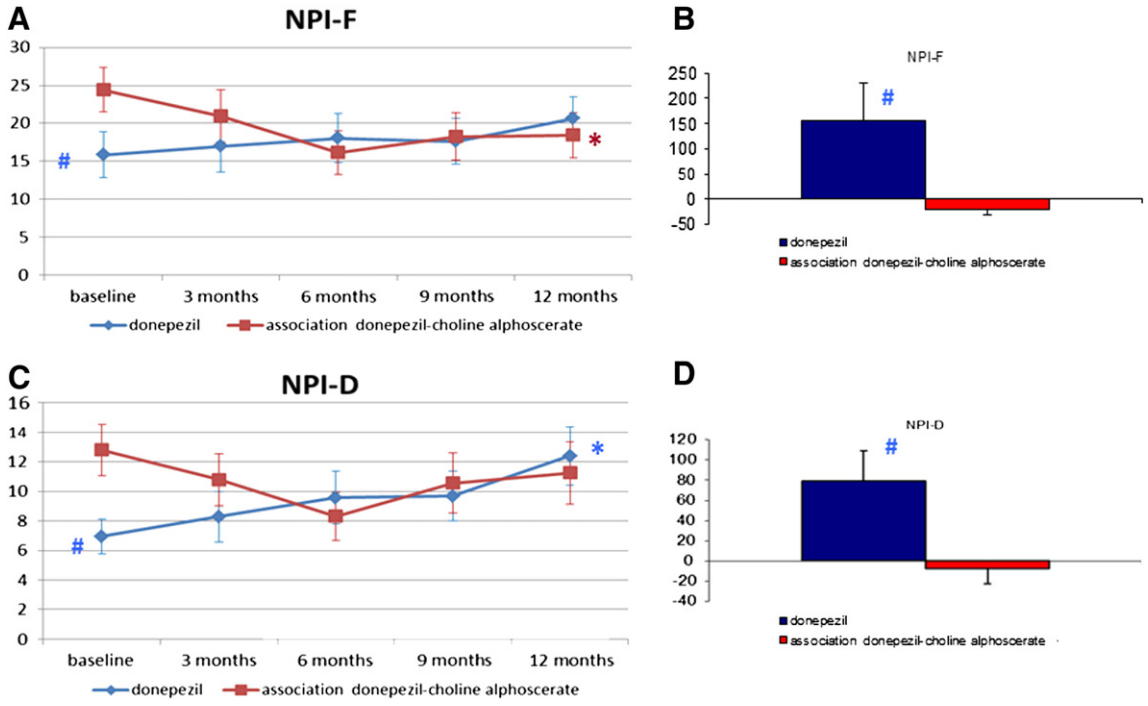

Fig. 3. Evaluation of behavioural symptoms by NPI-F (A) and NPI-D (C) tests. Panels B and D summarize the percentage variation of the parameters. Data are the means ± S.E.M. *p<0.05 vs. baseline; #p<0.05 donepezil vs. association therapy.

computerized case-report form for collection of medical data developed by the informatics section of Camerino University. The case report form, did include all the confidentiality and safety measures required for collecting medical data, and was placed in the WEB. Researchers of the two clinical units can access it as indicated in the Methods section. Hence, each centre could work independently with the advantage of an easy possible combination of the results obtained. This WEB-based procedure was followed also for economical reasons, being ASCOMALVA a clinical study of spontaneous generation. The good consistency of data obtained by the two centres, the possibility of easily analysing results obtained in real-time, suggests that WEB-based approaches for handling multicentre clinical trials may represent a good operational option for long time studies like those involving adult-onset dementia disorders. The supervising Centre provided to the units of Naples and Mantua the codes corresponding to the treatment of individual patients, according to the tables of general randomization of the study. It was therefore able to assess the progress of the trial at any time without the need of opening the blindness of the investigation.

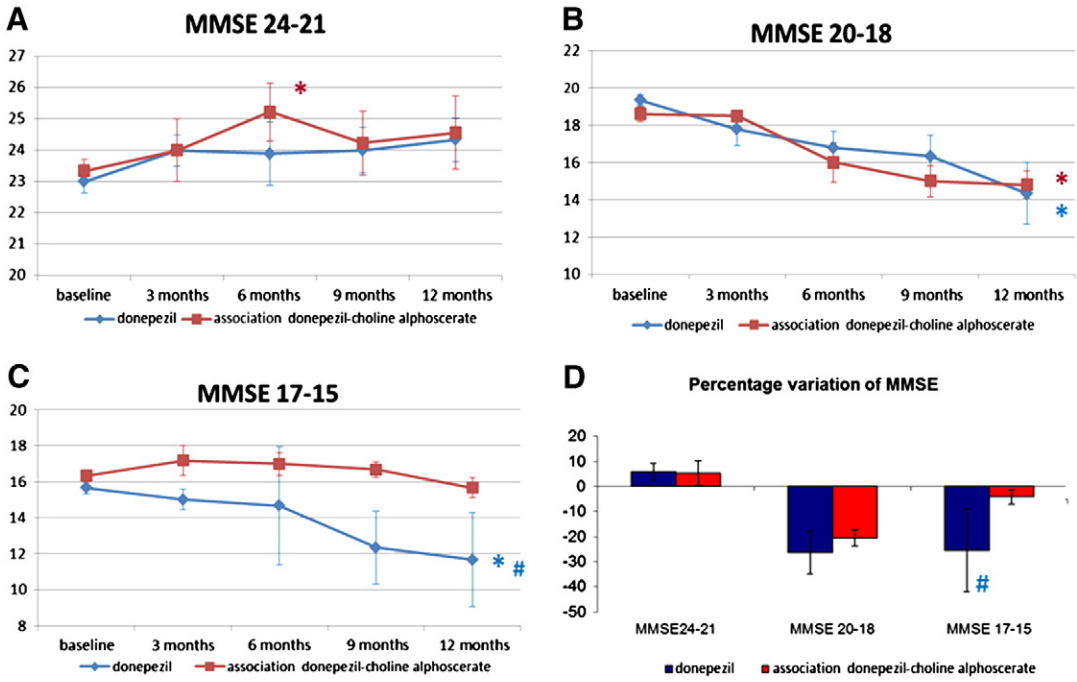

Fig. 4. Evaluation of the MMSE progression after stratification by MMSE at baseline: 25 > MMSE > 20 (A); 21 > MMSE > 17 (B); 18 > MMSE > 14 (C). Panel D summarises percentage variation of the parameter in the three groups. Data are the means ± S.E.M. * : p<0.05 vs. baseline; # p<0.05 donepezil vs. association therapy.

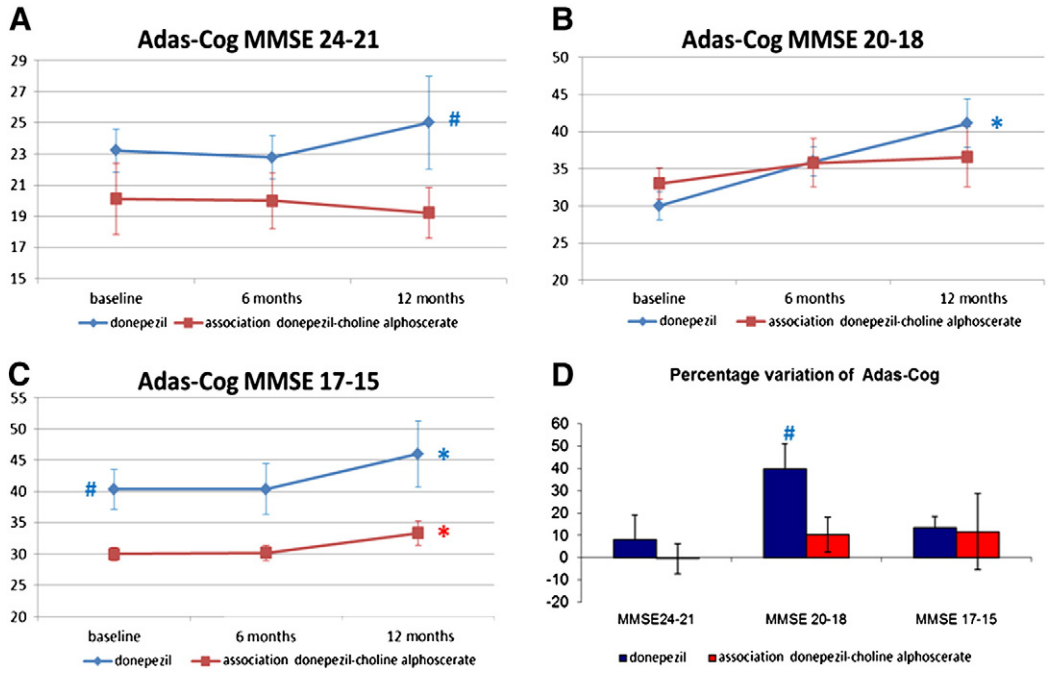

Fig. 5. Evaluation of the ADAS-Cog progression after stratification by MMSE at baseline: 25>MMSE>20 (A); 21>MMSE>17 (B); 18>MMSE>14 (C). Panel D summarizes percentage variation of the parameter in the three groups. Data are the means ± S.E.M. *p<0.05 vs. baseline; #p<0.05 donepezil vs. association therapy.

As mentioned above, cholinergic precursors and ChE-I represent pharmacotherapeutic strategies more extensively used for enhancing cholinergic neurotransmission in AD patients. Recent studies have also suggested a neuroprotective effect of ChE-I, which could slow the progressive evolution of the disease in question [16]. However, the benefits and cost/benefits of AChE/ChE-I are modest and for some studies of doubtful clinical significance [17]. 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-Is are not indicated [8]. The use of ChE-I at doses of greater efficiency (high) is also associated with side effects potentially relevant, including bradycardia which is relatively common.

Cholinergic precursors represent one of the first approaches to treat AD, but these compounds were investigated in clinical settings only sparsely compared to ChE-I and their effectiveness has been demonstrated only for some molecules [2]. Choline alphoscerate is between cholinergic precursors tested clinically probably the compound which has shown greater effectiveness in addition to good tolerability in patients with AD and VaD of mild to moderate degree [8]. Choline alphoscerate which easily crosses the blood–brain barrier

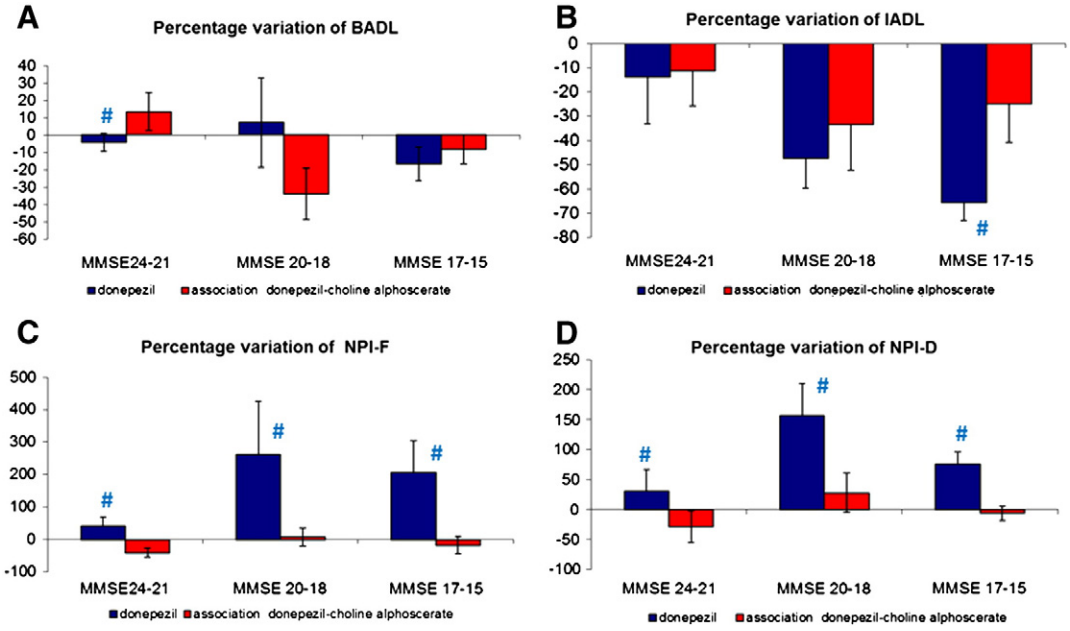

Fig. 6. Percentage variation of the parameters BADL (A), IADL (B), NPI-F (C), NPI-D (D) after stratification by MMSE at baseline. Data are the means ± S.E.M. #p<0.05 donepezil vs. association therapy.

probably acts as a donor of metabolically active choline in the brain and has demonstrated a neuroprotective effect in experimental animals with vascular brain injury.

Basal forebrain cholinergic structures involved cognitive activities are particularly sensitive to ischemia. This observation explains the marked cholinergic deficits reported in dementia in which neurodegenerative and vascular components are associated [5,18]. Based on these considerations, ASCOMALVA recruited patients with AD associated with vascular damage, a patient population characterized by a marked cholinergic hypofunction [19,20], which could benefit from a sustained cholinergic challenge such as that represented by the association of high doses of choline alphoscerate plus 10 mg/day donepezil.

Association of a cholinergic precursor (choline) plus a ChE-I such as tacrine or physostigmine was already tried, but with no apparent advantage compared to the treatment with ChE-Is alone [7]. This was not the case of ASCOMALVA, in which our interim data showed in one year observation that cholinergic association induced clinical effects more pronounced than the ChE-I alone and apparently slows down the progressive decline in therapeutic responsiveness which is common with long term administration of ChE-I.

5. Conclusions

AD patients with associated vascular injury probably represent the highest percentage of late-onset demented individuals as demonstrated by pathological, epidemiological and retrospective studies [3,18,21]. The consequences of neurodegenerative and vascular phenomena association in the pathophysiology and clinical course of AD are still under discussion. It is speculated that vascular lesions can make clinically apparent an underlying degenerative dementia, or that vascular and degenerative phenomena can progress in parallel [19]. Independently of these considerations AD patients with associated cerebrovascular injury constitute the largest group of demented elderly and probably, those in which the progression of disease is more aggressive [19].

Considering the lack of more effective therapies for AD at the moment, a reasonable strategy could be the combination/optimization of available treatments. The interim results obtained with ASCOMALVA suggest that the cholinergic association proposed could represent a therapeutic option to be considered in AD associated with cerebrovascular damage. Further larger scale investigations with an adequate time of observation are therefore needed to confirm this working hypothesis.

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