Meta-analysis of double blind randomized controlled clinical trials of acetyl-L-carnitine versus placebo in the treatment of mild cognitive impairment and mild Alzheimer's disease

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The efficacy of acetyl-L-carnitine (gamma-trimethyl-β-acetylbutyrobetaine (Alcar) in mild cognitive impairment (MCI) and mild (early) Alzheimer's disease (AD) was investigated with a meta-analysis of double-blind, placebo-controlled prospective, parallel group comparison studies of at least 3 months duration. The duration of the studies was 3, 6 or 12 months and the daily dose varied between studies from 1.5–3.0 g/day. An effect size was calculated to reflect the results of the variety of measures used in the studies grouped into the categories of clinical tests and psychometric tests. The effect sizes from the categories were integrated into an overall summary effect size. The effect size for the Clinical Global Impression of Change (CGI-CH) was calculated separately. Meta-analysis showed a significant advantage for Alcar compared to placebo for the integrated summary effect [$ES_{all\ scales} = 0.201$, 95% confidence interval (CI) = 0.107–0.295] and CGI-CH ($ES_{CGI\text{-}CH} = 0.32$, 95% CI = 0.18–0.47). The beneficial effects were seen on both the clinical scales and the psychometric tests. The advantage for Alcar was seen by the time of the first assessment at 3 months and increased over time. Alcar was well tolerated in all studies. *Int Clin Psychopharmacol* 18:61–71 © 2003 Lippincott Williams & Wilkins.

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Introduction

A series of randomized prospective double-blind placebo-controlled clinical trials in the literature on the use of acetyl-L-carnitine (gamma-trimethyl-β-acetylbutyrobetaine (Alcar) in mild cognitive decline and mild/moderate Alzheimer's disease (AD) have suggested that Alcar was effective in improving cognitive deficits or in delaying the progressive decline of AD patients.

Alcar is the most common natural short-chain acetyl carnitine ester of L-carnitine and is actively transported across the blood–brain barrier (Burlina *et al.*, 1989). L-carnitine functions physiologically as a shuttle between the cytoplasm and mitochondria for long-chain fatty acids, thereby participating in cellular energy production and in removal of toxic accumulations of fatty acids from mitochondria when several pathological conditions do not allow a complete oxidative catabolism (Lehninger, 1982; Pande *et al.*, 1986). Alcar also acts as a partial direct cholinergic agonist (Janiri *et al.*, 1991) and can be converted to acetylcholine (White and Scates, 1990). In animal studies, Alcar has been reported to protect central and peripheral nervous system synapses in neurodegenerative and ageing models (Villa *et al.*, 1986; Fariello *et al.*, 1988; Aureli *et al.*, 1990; Markowska *et al.*, 1990; Petruzzella *et al.*, 1992), to elevate nerve growth factor levels (Piovesan *et al.*, 1994; Taglialatela *et al.*, 1994) and to improve cognitive deficits in aged rats (Barnes *et al.*, 1990).

It is possible that an effect in mild cognitive impairment (MCI)/mild AD may be mediated via the biological effects of acetyl-L-carnitine. In some clinical studies, Alcar was efficacious already after 2–3 months of treatment (Agnoli, 1985; Bellagamba *et al.*, 1990; Herrmann *et al.*, 1990), which might indicate that its involvement in cellular energy production and its cholinergic functions are important for clinical efficacy. Patients with MCI/mild AD might be expected to benefit most from Alcar.

A majority of the early studies indicated that Alcar was effective, although a recent large, well-controlled study (Thal *et al.*, 1996) in mild/moderate AD patients lasting for 1 year did not demonstrate any difference from placebo. However, two other large placebo-controlled 1-year studies in mild/moderate AD patients (Spagnoli *et al.*, 1991; Rotmensch, unpublished data) showed a statistically significant superiority of Alcar over placebo on clinical as well as on psychometric measures. The latter two studies recruited mainly mild cases, whereas the majority of the cases in the study of Thal *et al.* (1996)

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were of moderate severity, as indicated by an average Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-Cog) of 25.9 at baseline.

The subjects in most of the earlier clinical studies were characterized as 'geriatric patients with mild cognitive impairment', as 'patients with progressive mental deterioration' or as 'patients with mild mental deterioration' or 'cognitively impaired individuals'. MCI is the generally used term for these patients, where cognitive impairment, particularly memory impairment, is beyond that expected in people of their age and educational level, but in whom a diagnosis of dementia is not appropriate. This term accords with the relatively recent diagnostic category in ICD-10 of mild cognitive disorder which defines a condition where cognitive decline is present but is not severe enough to reach criteria for dementia, delirium or organic amnesia syndrome. The decline in cognitive function may include memory disturbance, and learning and concentration difficulties.

We assessed the efficacy of Alcar in MCI and mild AD in a metanalysis of placebo-controlled, randomized double-blind studies lasting for at least 3 months because these patients were the most frequently studied in the past and because there are some reasons to believe that the effectiveness of Alcar may be more pronounced in milder cases.

Methods

Literature search

Studies for consideration were primarily identified through a literature search in Medline, Embase, Derwent Drug and Sci-Search from 1975 through 2001.

The search terms were 'Alcar' or 'L-acetyl-carnitine' or 'acetyl-L-carnitine' or 'levocarnitine' in the title and 'clinical trial' or 'cinical study' and 'controlled' or 'placebo' or 'double-blind'. A total of 30 papers were identified. Of these papers, 22 were study reports or overview articles on the use of Alcar in psychiatry/neurology and eight were related to other possible indications of carnitine derivatives.

The bibliographies of the publications were used to obtain additional related papers. This procedure was repeated four times.

A further publication search was made on the internal publication data bank of Sigma-Tau, the sponsor of most of the studies, and the bibliographies of additional publications found were examined.

Criteria for review and data collection

The criteria for inclusion in the meta-analysis were: (i) prospective, randomized, double-blind, placebo-controlled study with parallel groups; (ii) diagnosis compatible with MCI or mild AD, or sufficiently detailed description to indicate an equivalent diagnosis; (iii) duration of treatment at least 3 months; (iv) defined measures for drug effects on cognitive features or on the overall outcome; and (v) numeric data allowing calculation of the effect size (e.g. group means and standard deviations for differences from baseline or statistics that could be converted in a meta-analytic effect size). Studies that reported AD of different severity (mild, moderate, and severe) were acceptable if it was technically possible to single out the mild cases.

Some identified studies fulfilled all criteria except the necessary statistical information needed for the calculation of the effect sizes. In all these cases, supplemental information was available from the study reports of Sigma-Tau. One unpublished clinical study (identified as Rotmensch, protocol no. 09/0181-89) was retrieved from the Sigma-Tau database and considered in the meta-analysis based on the statistical report.

A total of 21 double-blind, placebo-controlled clinical trials in MCI or AD were identified in the 90 retrieved publications. Studies for the meta-analysis were primarily selected based on the diagnosis mentioned in the title and in the study inclusion–exclusion criteria. In a series of six studies, including 499 patients, the study inclusion–exclusion criteria gave a sufficiently detailed description to indicate a diagnosis equivalent to MCI. A cluster of six other studies consisted of 201 patients with mild AD. The remaining studies recruited patients with mild to moderate AD. For the four biggest of these studies (Spagnoli *et al.*, 1991; Thal *et al.*, 1996, 2000; Rotmensch, unpublished), the subpopulation of mild AD was abstracted from the database available at Sigma-Tau based on ratings of the Clinical Dementia Rating (CDR) (Morris, 1993) used in two studies (Thal *et al.*, 1996, 2000), on the Global Deterioration Scale (GDS) (Reisberg *et al.*, 1982) used in the study of Rotmensch, and the Blessed Dementia Rating Scale (BDS) (Blessed *et al.*, 1988) used in the study of Spagnoli *et al.* (1991). Patients with a CDR value equal to 0.5, a GDS score < 4, or a BDS value < 15 were included in the subpopulation.

It was not possible to separate mild from moderate cases of AD in three remaining small studies because we did not have access to individual case information. Baseline parameters indicated that these patients had predominantly mild AD. We decided to include these studies in the meta-analysis for the sake of fairness and to avoid any problems with selection bias. Unfortunately severity measures such as the GDS, CDR or Mini-Mental State Examination (MMSE) were not utilized in all of the studies.

The physician gave his impression of change at the end of the study in a total of 13 studies [Clinical Global Impression of Change (CGI-CH) in 12 studies and CIBIC (Clinician's Interview Based Impression of Change) in one study].

All the published studies included appeared between 1983 and 2000. The studies had durations ranging from 3 to 12 months and the dosages varied between studies from 1.5–3 g per day.

Publication bias

To assess the possibility of publication bias, the sample size was plotted on the *y*-axis and the effect size on the *x*-axis of a scatter plot and the funnel plot examined (Light and Pillemer, 1984). Because larger studies have more influence on the population effect size, smaller studies should be randomly scattered around the central effect size of larger studies, and some of the smaller studies are likely to show negative effect sizes (ES). The scatter should increase when study size decreases, giving rise to an inverted funnel appearance (Light and Pillemer, 1984; Egger *et al.*, 1997).

Meta-analytical method

Efficacy measures

Our meta-analysis focused on the disturbances of memory, attention, performance and higher intellectual functions suffered by patients with MCI and mild AD. The functions tested by Activities of Daily Living, Instrumental Activities of Daily Living scales and language tests are generally intact in this population and so were not considered in the meta-analysis. Mood scales were included in very few studies and these measures were therefore not included in the meta-analysis.

A total of 54 different scales were used to test efficacy in the studies. These included 12 clinical test batteries to test the severity of the disease, such as ADAS-Cog (Mohs and Cohen, 1988), Blessed-IMC (Test of Information, Memory and Concentration), MMSE (Folstein *et al.*, 1975), SCAG (Sandoz Clinical Assessment Geriatric)-total, CDR-sum of boxes, Benton-total, Gottfries-scale, 40 psychometric tests to test attention/performance (10 scales), memory (17 scales), higher intellectual functions (six scales), learning (three scales) and drawing (four scales), and two clinician ratings on disease change during the study (CGI-CH and CIBIC). The different efficacy measures were grouped into the categories 'clinical tests', which measured disease severity, and 'psychometric tests'. With the aim of describing all objective test results by one single efficacy parameter, all clinical and psychometric tests were finally integrated in a single summary category identified as 'all scales'. ES were calculated for each of these three categories and also for Clinical Global Impression, available in 13 studies.

The primary efficacy parameters were: (i) the integrated summary ES for all clinical tests (referred to as $ES_{\text{all scales}}$) used in the studies and (ii) the ES for clinicians judgment on efficacy (referred to as $ES_{\text{CGI-CH}}$), expressed by the CGI-CH (available in 12 studies) or CIBIC (available in one study).

A secondary analysis was made of the ES of different categories of psychometric test to investigate the efficacy profile of Alcar.

The effect sizes were calculated based on the results of the population having an assessment at every visit for the considered variable (completer dataset) because this was the population most frequently addressed in the available documents. In the remaining cases, the ES were based on the population present at the considered visit (i.e. sample sizes for baseline and other visits may differ) because these were the only available results. An overall dropout rate of 20% was observed and there was no imbalance in the rate between the two treatment groups.

ES estimation

As the data were all quantitative, the effect size analysis of Glass (1976) was used to combine the data from the independent trials.

Thus, in g studies, the generic study i ($i = 1, 2, \ldots, g$) has k scales. Within study i, the ES of a single scale j ($j = 1, 2, \ldots k$) was defined as the difference between the mean changes from baseline to end-point of the two treatment groups divided by the SD of the change score. Hedges's d formula was applied to obtain an unbiased estimator of the ES (Hedges and Olkin, 1985):

$$d_{ij} = \frac{Y_{ij}^{ALC} - Y_{ij}^{PLA}}{SD_{ij}} * \left(1 - \frac{3}{4 * N_{ij} - 9}\right) \qquad (1)$$

where $Y_{\text{ij}}^{\text{ALC}}$ = mean change (last value – baseline) of Alcar group in study i and scale j; $Y_{\text{ij}}^{\text{PLA}}$ = mean difference (last value – baseline) of placebo group in study i and scale j; SD_{ij} = average SD of the two treatment groups in study i and scale j; and N_{ij} = sum of sample sizes of the two groups in study i and scale j. For CGI-CH, Y equals the last value because CGI-CH, by definition, is the clinician's impression of change related to the baseline status.

An increase in score signifies amelioration for some of the scales used and deterioration for others. We decided that a positive ES should always indicate therapeutic superiority of the drug over placebo and therefore multiplied the ES by –1 where appropriate.

For studies that used multiple scales within a specific category, the effect sizes by each individual scale were estimated with Equation (1) and were then pooled to produce a common ES for the study and the category, using the Hedges's estimator for correlated data:

$$\delta_i = w_{i1}d_{i1} + \ldots . + w_{ik}d_{ik} \qquad (2)$$

where k = number of scales in study i for the considered category; d_{ij} = Hedges's d unbiased estimate of the ES in study i and scale j; and w_{ij} = optimal weights depending on the variance–covariance structure among the scales.

This is in effect a weighed linear combination of the scale effect sizes, where weights are based on the variance-covariance structure among the scales. The required correlation coefficients between each pair of the scales were only sparsely available and a fixed correlation of 0.5 was assumed for all the scales.

The majority of studies had a complete dataset and including or excluding the scales with incomplete data did not affect the results. The results are presented on the available data and no method for dealing with missing data was applied for computing the Hedges's δ estimates.

In order to obtain a pooled estimate of the effect size for each category in all studies, the effect sizes of the category were combined, using the Hedges's $d+$ estimate for independent data:

$$d+ = w_1^*d_1^* + \ldots . + w_g^*d_g^* \qquad (3)$$

where g = number of considered studies; d_i^* = for studies with a single scale, it is Hedges's d estimate (see Equation (1)); for studies with multiple scales within the considered category, it is the Hedges's δ estimate (see Equation (2)):

$$w_i^* = \frac{1}{\mathrm{var}(d_i^*)} \Big/ \sum_{i=1}^{g} \frac{1}{\mathrm{var}(d_i^*)}$$

In this weighed linear combination of the study effect sizes, weights are based on variances and sample sizes with greater weight given to bigger studies (Hedges and Olkin, 1985).

The variances and the 95% confidence intervals (95% CI) (assuming the normal approximation) were computed for the effect sizes of each category, both at each individual study level (i.e. Equations (1) or (2), depending on the number of scales) and at the combined study level (i.e. Equation (3)) (Hedges and Olkin, 1985).

Homogeneity

Homogeneity of effect sizes across scales within a study or across studies was tested with an approximate chi-square test for independent data using the formula:

$$Q_{ind} = \sum_{i=1}^{g} \frac{(d_i^* - d+)^2}{\mathrm{var}(d_i^*)}$$

and for correlated data, using an assumed correlation of 0.5.

Results

The meta-analysis was based on 21 identified studies in MCI and/or mild AD. A total of 1479 patients were included in the studies. A total of 1204 patients had end study assessments and were available for analysis (591 in the Alcar groups and 613 in the placebo groups). The number of patients in the individual studies varied from 12 to 183 patients. The demographic data show the typical pattern for a MCI/mild AD population (Table 1). There were more females (59%) than males (41%), the age at the start of the study was 71.9 ± 6.5 years. The age of onset is mentioned in only six studies and varied from 2 to 3.5 years before the start of the study. The educational level was given in 11 studies. Patients in the USA studies had an average 13.9 years schooling whereas the Italian patients had an average of only 4.9 years. The clinical baseline condition was described with 12 different test batteries, but at least one of the following scales was used in all but three studies: ADAS-Cog (Mohs and Cohen, 1988), BDS (Blessed *et al.*, 1988), MMSE (Folstein *et al.*, 1975) or GDS (Reisberg *et al.*, 1982). The

Table 1 **Demographics and baseline characteristics**

		Alcar	Placebo
Sex	Females	58%	59%
	Males	42%	41%
Age			
Number of studies 21	Weighted mean ± SD	71.8 ± 6.6	72.0 ± 6.3
	(min – max)	(42–95)	(47–97)
BDS			
Number of studies with BDS: 9	Weighted mean ± SD	8.4 ± 3.24	8.6 ± 3.39
GDS			
Number of studies with GDS: 4	Weighted mean ± SD	3.2 ± 0.38	3.2 ± 0.40
MMSE			
Number of studies with MMSE: 14	Weighted mean ± SD	19.0 ± 3.4	19.1 ± 3.4
ADAS-Cog			
Number of studies with ADAS-Cog d: 4	Weighted mean ± SD	18.5 ± 7.6	16.5 ± 6.6

Weights are given by the number of patients per study having baseline assessment for the corresponding variable. Weighted SD is the square root of the weighted mean of variances available by study

BDS was used in nine studies, the GDS in four studies, the MMSE in a total of 14 studies and the ADAS-Cog in four studies. Table 1 shows the baseline characteristics of the patients.

In all studies, patients were randomized to treatment to achieve equal groups. The studies were performed in Italy, Great Britain, Germany and the USA. Most were multicentre studies.

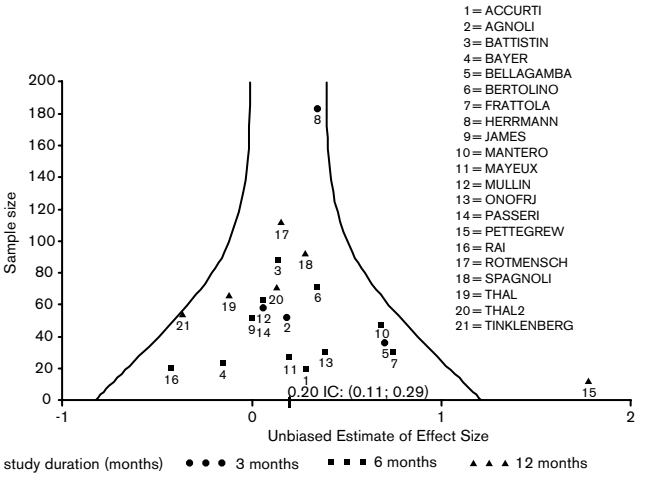
In 11 studies, treatment duration was for 6 months; in six studies for 1 year; and in four studies for 3 months. In studies lasting for 1 year, patients were also examined at months 3 and 6 and, in the 6-month studies, patients were also frequently checked at month 3. The last available results were used to calculate the effect sizes for the primary analysis.

Alcar was well tolerated in all studies. The reported side-effects and adverse events were similar in nature and severity in the two treatment groups. Gastrointestinal events (nausea, abdominal discomfort, vomiting) insomnia, agitation and increased appetite were reported somewhat more frequently for Alcar in some studies. Early dropouts were well balanced between the two treatment groups and most often were a consequence of intercurrent diseases.

Testing for publication bias

The total sample size of each study plotted against the effect size $ES_{all\ scales}$ is shown in Fig. 1. The funnel plot does not provide any evidence for publication bias: the results are symmetrically scattered around the average population effect size in the form of an inverted funnel, and studies with a small sample size are apparently as likely to have a negative or positive outcome. On the other hand, inspection of the funnel raises the possibility that study 15 (Pettegrew *et al.*, 1995) is so much outside of the funnel that it may be an outlier. The $ES_{all\ scales}$ was therefore calculated with and without this study.

Fig. 1



Funnel plot: Sample size against $ES_{all\ scales}$.

Testing for homogeneity.

For. the summary category 'all scales' there is a marginally significant heterogeneity in the effect sizes across studies. This is mainly due to the study of Pettegrew *et al.* (1995) which had a very high ES and, when this study is excluded, there is no significant heterogeneity in the effect sizes across studies. The pooled effect sizes ($ES_{all\ scales}$), including and excluding this study, are nearly identical.

Looking at the components of $ES_{all\ scales}$, the battery of psychometric tests shows no heterogeneity between studies but the clinical tests do. The heterogeneity persisted even when the study of Pettegrew *et al.* (1995) was excluded.

Heterogeneity is also found in the judgment CGI-CH. This is mainly due to two studies, one with an outstanding negative, the other with an outstanding positive $ES_{CGI\text{-}CH}$ (Table 2).

Combined effect size

The $ES_{all\ scales}$ that integrates all clinical and psychometric tests was calculated for all 21 studies. $ES_{all\ scales}$ combines the results of 4.8 scales on average (1–13), with up to four clinical scales and from 1–11 psychometric tests.

The integrated summary ES ($ES_{all\ scales}$) pooled over all 21 studies accounts for 0.201 (95% CI 0.107–0.295). This ES varies between –0.43 and 1.77 among the individual studies. A total of 17 out of 21 studies show positive estimates for $ES_{all\ scales}$. No significant correlation was found between $ES_{all\ scales}$ and educational level ($r = -0.18$).When the study of (Pettegrew *et al.*, 1995) is excluded, a significant overall effect for Alcar is still obtained (i.e. 0.191 with 95% CI 0.10–0.29) (Table 3). The ES based on objective measures and the ES based on CGI-change are highly correlated ($r = 0.9$) (Fig. 2).

The ES for clinicians impression of change ($ES_{CGI\text{-}CH}$) varied in the individual studies from –0.74–1.22 (Table 3). The pooled ES was 0.32 (95% CI 0.18–0.47).

Analysis of psychometric test profile.

The 40 different psychometric tests were grouped into five categories: attention/performance (10), memory (17), learning (3), drawing (4) and higher intellectual functions (6). Table 4 shows ES for these categories.

Table 2 Testing homogeneity

Main parameter	Subgroups ES	Chi squared	d.f.	*P*
Summary $ES_{all\ scales}$ including all tests		33.65	20 (all studies)	0.03
		27.02	19 (study by Pettegrew *et al.*, 1995 excluded)	NS
	Clinical tests	51.48	19	<0.001
		45.49	18 (study by Pettegrew *et al.*, 1995 excluded)	<0.001
	Psychometric tests	15.65	14	NS
	Performance/attendance	8.09	12	NS
	Memory	14.85	9	NS
	Intellectual functions	13.41	6	0.04
	Learning	7.76	4	NS
	Drawing	4.84	5	NS
Summary $ES_{CGI\text{-}CH}$ for CGI-CH		37.11	12	<0.001

Table 3 Effect size (ES) at study end for the integrated efficacy summary variable ($ES_{all\ scales}$) and $ES_{CGI\text{-}CH}$

Study Id	Reference	Study duration (months)	Daily dose (g)	Alcar, *n*	Placebo, *n*	Total, *n*	Scales by study	$ES_{all\ scales}$	$ES_{CGI\text{-}CH}$
ACCURTI	(Giuliani *et al.*, 1990)	6	1.5	10	9	19	7	0.288	0.61
AGNOLI	(Agnoli, 1985, 1994)	3	2	26	26	52	6	0.184	
BATTISTIN	(Battistin *et al.*, 1989)	6	2	44	44	88	9	0.137	
BAYER	(Bravi *et al.*, 1994; Bayer, 1994)	6	2	12	11	23	3	−0.154	−0.74
BELLAGAMBA	(Bellagamba *et al.*, 1990; Bellagamba, 1991)	3	3	17	19	36	6	0.703	0.88
BERTOLINO	(Bertolino and Papagno, 1983)	6	1.5	34	37	71	5	0.348	
FRATTOLA	(Bassi *et al.*, 1988; Frattola *et al.*, 1988; Frattola, 1991)	6	1.5	15	15	30	13	0.751	1.22
HERRMANN	(Herrmann, 1988; Herrmann *et al.*, 1990)	3	1.5	92	91	183	1	0.346	0.52
JAMES	(Livingston *et al.*, 1991; James, 1992)	6	2	26	25	51	1	0.001	0.29
MANTERO	(Mantero *et al.*, 1989; Mantero, 1991)	6	2	23	24	47	2	0.686	1.16
MAYEUX	(Sano *et al.*, 1992)	6	2.5/3	13	14	27	10	0.200	
MULLIN	(Bravi *et al.*, 1994; Mullin, 1994)	6	2	30	32	62	2	0.062	−0.06
ONOFRJ	(Onofrj, 1992; Bravi *et al.*, 1994)	6	3	15	15	30	5	0.390	1.02
PASSERI	(Cucinotta *et al.*, 1988; Passeri *et al.*, 1990)	3	2	30	28	58	7	0.058	
PETTEGREW	(Pettegrew *et al.*, 1995)	12	3	7	5	12	2	1.775	
RAI	(Rai, 1989, Rai *et al.*,1990)	6	2	7	13	20	4	−0.426	
ROTMENSCH	(Rotmensch, 1993)	12	3	51	61	112	2	0.157	0.24
SPAGNOLI	(Spagnoli *et al.*, 1991)	12	2	42	50	92	6	0.283	
THAL, 1996	(Toth *et al.*, 1993; Thal *et al.*, 1996)	12	3	33	33	66	4	−0.119	0.07
THAL, 2000	(Thal *et al.*, 2000)	12	3	34	37	71	4	0.131	−0.09
TINKLENBERG	(Bravi *et al.*, 1994)	12	2–3	30	24	54	2	−0.364	−0.29
				Total *n* Alcar	Total *n* placebo	All cases	Mean	Pooled ES (95% CI)	Pooled ES (95% CI)
				591	613	1204	4.8	0.201 (0.107–0.295)	0.32 (0.18–0.47)

The global ES for the psychometric tests, excluding drawing and learning used in very few studies, showed a significant effect for Alcar.

Time course.

Efficacy. was measured based on objective scales at month 3 as well as on month 6 in a total of 16 studies (Table 5).

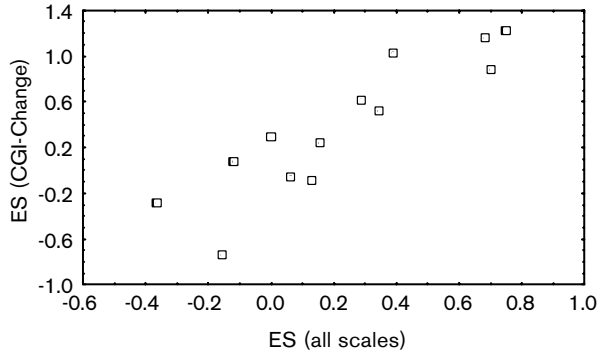
Clinical and psychometric tests, as well as CGI-CH, show significant positive effect sizes after 3 months of treatment (Table 5). The ES for clinical scales and for psychometric tests increase with time, whereas the clinicians rating is somewhat less positive but still significant after 6 months. Results on clinical tests and CGI-CH are heterogeneous among studies but the results of the psychometric tests are homogeneous.

Table 4 **Effect size (ES) at study end for different categories of psychometric tests**

Psychometric test batteries	No of different tests	No of studies with tests	ES Unbiased estimate	95% Lower limit	95% Upper limit
Global ES for psychometric tests	40	15	0.214	0.101	0.327
			(0.255)[a]	(0.136)[a]	(0.373)[a]
Attention/performance	10	13	0.221	0.090	0.352
Memory	17	10	0.290	0.132	0.449
Intellectual functions	6	7	0.373	0.173	0.573
Drawing	4	6	0.054	−0.162	0.270
Learning	3	5	0.066	−0.188	0.319

[a]Global ES for Attention/performance, Memory and intellectual functions but without drawing and learning.

Fig. 2



Correlation between objective and subjective outcome measures (correlation coefficient = 0.90).

Table 5 **ES and relevant homogeneity tests over time**

	Month 3		Month 6	
	ES	Homogeneity test	ES	Homogeneity test
Clinical tests	0.16*	<0.001	0.22*	<0.001
Psychometric tests	0.14*	0.718	0.16*	0.379
CGI-CH	0.29*	0.047	0.22*	<0.001

*$P < 0.05$.

Table 6 **Daily dose of Alcar**

Daily dose of Alcar	No of subjects	%
1.5	151	25.5
2	270	45.7
3	170	28.8
1.5	151	100

Efficacy and dose

No significant correlations between the two outcome measures and the daily dose were found (Spearman *r*). This is not surprising because the dose range was narrow and varied in the 21 studies from 1.5–3 g per day (Table 6).

Discussion

Alcar has been used to treat cognitive deficits of elderly patients for many years. There is an extensive body of studies suggesting the efficacy for Alcar in improving cognitive deficits or delaying the progressive decline of AD patients, although a recent large study did not establish a difference from placebo. Our meta-analysis of placebo-controlled studies found a significant advantage for Alcar compared to placebo, and this was seen both in a composite measure of effect size on clinical and psychometric assessment scales and on the clinicians' assessment of improvement. Our positive results are important in providing a comprehensive perspective on the therapeutic effect of Alcar.

Caution is needed in interpreting the results of meta-analyses of published studies which are beset by methodological difficulties and a potential for bias. In our study, strenuous efforts were made to address these problems.

In this type of analysis, the selection of studies for inclusion is sometimes criticised as being incomplete or biased. There is a particular concern that studies with no effect are less likely to be published than studies with a (statistically significant) positive outcome. This could result in a misleading meta-analysis because the published studies would not be representative of all studies that have been carried out.

We adopted stringent search and inclusion criteria and considerable effort was made to identify all studies meeting the criteria: placebo-control, use of a diagnosis in accord with MCI or mild AD, study duration at least 3 months, use of scales to assess effect, numeric data published that could be converted to a meta-analytic effect size. The search was not limited to English language publications. In the initial literature search, a number of studies were identified for potential inclusion but had only been published in abstract form. A further search was therefore made with the assistance of the pharmaceutical company who was the sponsor of most of the studies so that full data from the study reports, or in four cases the original database, could be used (Spagnoli *et al.*, 1991; Thal *et al.*, 1996, 2000; Rotmensch).

We also tested our selection of studies for bias by use of a funnel plot to see if a multiplicity of smaller studies carried a disproportionate weight in the results. No evidence of bias was found. This analysis indicated that one study with a particularly positive effect might have been an outlier. However, exclusion of this study did not affect the result.

Although several of the trials were conducted some years ago, their quality was judged to be reasonable. All studies were double-blind, randomized, placebo-controlled with parallel group design. The completer population was the basis for the meta-analysis because this was the population presented in most of the documents, but the number of early withdrawals was small and similar in the two treatment groups.

Another problem with meta-analyses is the often widely differing methodologies used in the studies under consideration. The studies analysed here were similar in design, indication and route of administration, but differed in choice of test instruments, clinical environment, concomitant activities, daily dose and duration of treatment, all of which might lead to heterogeneous results. We therefore tested for homogeneity before pooling the individual study ES to derive a common ES. The marginal heterogeneity detected in the meta-analysis was mainly attributable to one study (Pettegrew *et al.*, 1995), which had extremely positive results and was identified as a possible outlier. However, when the analysis was repeated excluding this study, the calculated effect sizes were nearly identical, the weight of this small study was small, and the heterogeneity of the results was no longer obtained. It is possible that certain aspects of the design in this study may have influenced the results. In addition to the usual clinical program, the patients in this study were repeatedly tested with P-31 magnetic resonance spectroscopic measures, which may have had an unidentified influence on patient selection or patient motivation.

The meta-analysis covered a time span of 20 years, during which the methodology for investigating MCI and AD has developed considerably and some pivotal scales in use currently were not available to the early studies. A possible criticism of the meta-analysis is the possible inclusion of some subjects who may have had moderate dementia. In the majority of studies, the diagnostic descriptions were sufficient to determine that patients fulfilled our criteria for MCI or mild AD. In order to avoid selection bias, three small studies for which there was insufficient information to separate mild and moderate cases were included on the assumption that the inclusion of any unidentified moderate AD patients would reduce any positive effect of Alcar.

Because a wide variety of test measures was used in the studies, we categorized them for analysis into clinical and psychometric, the latter being subsequently subcategorized to explore the pattern of effect of Alcar.

Some studies used more than one test in a category. We therefore calculated, for each category, a common effect measure by pooling the results of a study within the categories. It could be argued *a priori* that the scales within a category do not measure different constructs and that they correlate each with the other. Methodological studies have shown that the correlation coefficients for test batteries within a category reach or exceed the level of 0.7 (Erkinjuntti *et al.*, 1988; Zhang, 1991; Villardita and Lomeo, 1992; Berg *et al.*, 1993; Elwan *et al.*, 1994; Kincaid *et al.*, 1995; Vajdicková *et al.*, 1995; Brodaty and Moore, 1997; Burkart *et al.*, 1998; Ihl *et al.*, 1999; Chu *et al.*, 2000; Demers *et al.*, 2000). In order to obtain a combined estimate of the effect size for the different scale categories within the studies, we pooled the estimates of the individual scales to a common ES, using a rather conservative assumption of 0.5 for the correlation coefficient for the intra-category ES where specific study information was not available. We then calculated an ES that pools all test results inside a study ($ES_{\text{all scales}}$). This ES combined the results of minimally 2 and maximally 13 scales within a study.

The advantage of this procedure is that it avoids biased decisions on the selection of the efficacy parameters to be used for meta-analysis and presents a single efficacy parameter. This approach is justified by the fact that the different tests were originally selected to investigate different aspects of MCI/mild AD, and it is fair to assume that the different tests share principally a common effect. This type of global measure from multiple tests provides a solution to assessment of treatment efficacy where no single outcome measure is sufficient, as is clearly the case in MCI/mild AD. None of the individual scales used in the studies would describe all relevant aspects of efficacy. Previously, studies have been criticised for reporting favourable results from isolated scales making the results difficult to interpret. CGIs for severity or change during treatment attempt to overcome this weakness and give the clinician the chance to judge the overall treatment success, taking clinically relevant aspects into account. In neuropsychiatry, the CIBIC is now often used to provide a more balanced and consistent global judgment, but interrater variability can still be a problem (Boothby *et al.*, 1995). We considered it reasonable to unify the multiple endpoints in one single variable to provide an estimate of the effect of treatment. A global test based on objective measures such as $ES_{\text{all scales}}$ checks for a favourable treatment outcome and is largely independent from subjective influences.

There were some differences between the $ES_{\text{all scales}}$ which represents a global test based on objectives measure and a global test based on the clinician's judgment ($ES_{\text{CGI-CH}}$). The two main efficacy variables $ES_{\text{all scales}}$ and $ES_{\text{GGI}-\text{CH}}$, pooled over all studies, both demonstrate the efficacy of Alcar and correlate strongly ($r = 0.9$). However, the results on the $ES_{\text{all scales}}$ were homogeneous whereas results based on CGI-CH were not. This lack of homogeneity may be a rating problem or may reflect clinicians' differing attitudes. The $ES_{\text{CGI-CH}}$ is on average higher then $ES_{\text{all scales}}$ with a tendency to a more pronounced view in a positive as well as in a negative direction. Both measures were significant by month 3, with $ES_{\text{GGI}-\text{CH}}$ having reached its highest point whereas $ES_{\text{all scales}}$ reaches its maximum only after 6 months of treatment. A total of four out of the 21 studies showed negative $ES_{\text{all scales}}$ whereas the $ES_{\text{all scales}}$ of all the other studies were positive. If we exclude the positive outlier (Pettegrew *et al.*, 1995), we find a normal distribution of the the $ES_{\text{all scales}}$ of the individual studies (skewness = 0.009, kurtosis = 0.005). The educational level of the patients participating in the analysed studies was much higher in the US studies than in the Italian studies. This reflects differences in the national educational laws and is in line with surveys on the educational level in different countries (Molarius *et al.*, 1998). Education is correlated with cognitive status assessment, less education being associated with lower scores on MMSE (Uhlmann and Larson, 1991; Jagger *et al.*, 1992; Tombaugh and McIntyre, 1992; Launer *et al.*, 1993; Freidl *et al.*, 1996; Magni *et al.*, 1996; Ostrosky Solis *et al.*, 1999). This may explain the low MMSE baseline levels in patients who did not otherwise have severe dementia scores in some Italian studies.

The relationship between the decline in cognitive performance and educational level is still contentious. It was recently reported that the level of educational attainment did not influence the functional decline of the MMSE (Jones and Gallo, 2001), whereas other studies have found that lower education was predictive of decline on the MMSE and on tests of language and knowledge, but not on tests of cognitive speed, memory or reaction time (Christensen *et al.*, 1997). Individuals with higher levels of education may show relative stability over time on language and secondary memory tasks but deteriorate as rapidly as individuals with low education on visuospatial tasks (Ritchie *et al.*, 1997). Memory and language functions have been shown to be more resistant to decline in the high-education group, while attention, implicit memory and visuospatial skills decline irrespective of education level (Leibovici *et al.*, 1996). We found no correlation between $ES_{\text{all scales}}$ and the educational level, although this is not surprising because an influence of educational attainment on the decline in cognitive performance would take place in the Alcar treated patients as well as in the placebo group and would therefore be automatically eliminated during the calculation of the effect sizes.

The secondary analysis of the psychometric tests shows highest efficacy for Alcar on memory and intellectual functions. This is important because memory deficits and disturbed intellectual function are the most pronounced deficits in MCI.

The great majority of the patients were treated in a narrow dose range, with daily doses of 1.5–2.0 g. The lack of significant dose response effect is therefore not surprising. The pharmacokinetics of Alcar are complex, similar to other naturally occurring substances and include partial prehepatic metabolism, first liver pass effect and varying bioavailability.

Alcar was well tolerated in all studies. Adverse events were unspecific, mostly mild and were found with a similar frequency with Alcar and placebo. We therefore think it unlikely that the blind was broken by the appearance of adverse events. The lack of increased side-effects in the Alcar group confirms Alcar as a safe well-tolerated medication.

In summary, the positive effect in this meta-analysis of Alcar compared with placebo in improving mild cognitive impairment or preventing deterioration suggests that Alcar should be considered for treatment of these important conditions.

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