A 28-week, double-blind, placebo-controlled study with Cerebrolysin in patients with mild to moderate Alzheimer's disease

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Cerebrolysin (Cere) is a compound with neurotrophic activity which has been shown to be effective in the treatment of Alzheimer's disease (AD) in earlier trials. The efficacy and safety of repeated treatments with Cere were investigated in this randomized, double-blind, placebo-controlled, parallel-group study. One hundred and forty-nine patients were enrolled (76 Cere; 73 placebo). Patients received i.v. infusions of 30 ml Cere or placebo 5 days per week for 4 weeks. This treatment was repeated after a 2-month therapy-free interval. Effects on cognition and clinical global impressions were evaluated 4, 12, 16, and 28 weeks after the beginning of the infusions using the Clinical Global Impression (CGI) and the Alzheimer's Disease Assessment Scale – cognitive subpart (ADAS-cog). All assessments, including the 28-week follow-up visit were performed under double-blind conditions. At week 16, the responder rate of the Cere group was 63.5% on the CGI, compared to 41.4% in the placebo group ($P < 0.004$). In the ADAS-cog, an efficacy difference of 3.2 points in favour of Cere was observed ($P < 0.0001$). Notably, improvements were largely maintained in the Cere group until week 28, 3 months after the end of treatment. Adverse events were recorded in 43% of Cere and 38% of placebo patients. Cere treatment was well tolerated and led to significant improvement in cognition and global clinical impression. A sustained benefit was still evident 3 months after drug withdrawal. Int Clin Psychopharmacol 16:253–263 © 2001 Lippincott Williams & Wilkins

INTRODUCTION

Alzheimer's disease (AD) is one of the commonest diseases of the elderly. Ageing is the most important risk factor for AD. The prevalence of AD increases from 3% in the group aged 65–75 years to 15% in people over 85 years. Delaying the onset of the disease by 5 years would decrease the prevalence of the disease by 50% within one generation (Khachaturian, 1992).

The main therapeutic approach to AD has been cholinergic transmitter replacement which led to the development of cholinesterase inhibitors. Overall, however, their efficacy has been modest at best and they provide only symptomatic benefit (Gauthier, 1999). Thus, stabilization strategies aiming at delaying disease progression are being investigated. Several avenues of research are being explored, including anti-oxidants, anti-inflammatory drugs and neurotrophic agents (Gray and Gauthier, 1999).

Among the latter, nerve growth factor (NGF) has been considered since this protein plays an important role in the survival of cholinergic neurons. It prevents cholinergic degeneration after lesions of the septohippocampal pathways or basal forebrain nuclei (Hagg *et al.*, 1989; Stromberg *et al.*, 1990; Koliatsos *et al.*, 1991) and counteracts lesion dependent cognitive deficits in rats (Pallage *et al.*, 1986; Calamandrei *et al.*, 1991; Fischer *et al.*, 1991; Frick *et al.*, 1997). Therapeutic trials in patients have been considered (Tuszynski and Gage, 1991; Beck and Hefti, 1993) with the limitation that NGF does not cross the blood–brain barrier. A first clinical trial with intracerebroventricular application of NGF in AD (Seiger *et al.*, 1993) did not provide clear results, probably due to the invasive application method.

The drug Cerebrolysin (Cere) is a peptide preparation produced by biotechnological methods, using a standardized proteolytic breakdown of purified porcine brain proteins. It consists of approximately 25% biologically active low molecular weight peptides and amino acids. One ml of Cere contains 9 mg of peptides and the consistent qualitative and quantitative composition of the compound is ensured by rigorous quality control procedures, including amino acid analysis and high-performance liquid chromatography peptide mapping.

Satou *et al.* (1993, 1994) reported that Cere exerts a neurotrophic activity similar to NGF on dorsal root ganglia. Control experiments using antibodies against NGF showed that Cere's activity could not be blocked, suggesting that the active components of Cere are different from endogenous NGF. In a model of fimbria fornix transection, Cere prevented the degeneration of medial septal cholinergic neurons. Protection was achieved after peripheral injection indicating that the small molecules are able to penetrate blood–brain barrier in pharmacodynamically significant amounts (Akai *et al.*, 1992). Comparison of i.c.v. versus intraperitoneal injections supported this finding (Gschanes *et al.*, 1997). Cere treatment also improved fimbria fornix transection induced cognitive deficits in rats in a spatial orientation task. Its peripheral application was more effective than the i.c.v. infusion of NGF and, in contrast to NGF, it improved not only memory retrieval, but also acquisition of new information (Francis-Turner *et al.*, 1996; Valouskova and Francis-Turner, 1998). Moreover, in apolipoprotein (Apo)E knockout mice which show early neuronal degeneration, Cere ameliorated the cognitive deficits (Masliah *et al.*, 1999). The 4-week treatment increased the synaptic density and exhibited morphological effects suggestive of a normalization of neuronal cytoarchitecture com-

pared to deficits in control ApoE knock-out mice. Significant improvements of spatial learning and memory were also achieved after 4 weeks of treatment in 24-month-old rats (Gschanes and Windisch, 1998), which was associated with significant increases in synaptic density in the hippocampal formation as well as in the entorhinal cortex (Reinprecht *et al.*, 1999).

In a randomized, placebo-controlled, double-blind clinical trial investigating the effects of a 4-week treatment with 30 ml of Cere in 120 patients suffering from mild to moderate AD, significant improvements in cognition, global rating, and activities of daily living were reported (Ruether *et al.*, 1994). Six months after cessation of treatment, these improvements were at least partially maintained (Ruether *et al.*, 2000). Panisset *et al.* (2000) reported the results of a randomized, placebo-controlled, double-blind clinical trial on 192 AD patients. Significant improvements in clinical global impression (CIBIC +) were observed in Cere-treated patients and a trend for improvement of activities of daily living was noted. Remarkably, these effects were observed 2 months after the end of active treatment, suggesting a stabilizing effect of Cere.

Our current clinical trial was designed to confirm and extend the efficacy and safety data from earlier trials. The administration protocol, an active treatment period of 4 weeks, with one daily short-term infusion of 30 ml of Cere or placebo, five times a week, was replicated from previous studies. After a treatment-free interval of 8 weeks, however, this regimen was repeated, accounting for a total of 40 Cere or placebo infusions. The confirmatory analysis of the primary parameters was set to be at the week 16 visit, after the end of active treatment. A week 28 visit was performed to investigate a potential stabilizing effect of Cere.

METHODS

Study design

This was a 7 month, randomized, double-blind, placebo-controlled, parallel-group study conducted at nine investigational sites, hospitals and ambulances in Germany and Austria, with 149 patients being enrolled in two groups: Cere 30 ml ($n = 76$) and placebo ($n = 73$). Patients were screened for study entry within 14 days of the baseline visit, at which time eligible patients were randomized into the study. Thereafter, patients received i.v. infusions of either Cere or placebo 5 days per week for 4 weeks. This regimen was repeated after 2 months treatment-free interval. The week 4 visit was sched-

uled 4 weeks after the baseline examination, within 8 days of the end of the first treatment. The week 12 visit was scheduled 12 weeks after baseline, the week 16 visit was scheduled 16 weeks after baseline, within 8 days from the end of the second treatment. A follow-up examination (week 28) was scheduled 28 weeks after baseline, 3 months after the end of active treatment. The study was conducted under double-blind conditions until after the week 28 follow-up visit, and thus, followed current guideline recommendations requiring a 6-month double-blind study period.

Patient population
Patients were evaluated by clinical interview, mental status assessment, physical and neurological examination, neuroimaging and laboratory studies. Male and female outpatients aged 50–85 years were eligible for this study if they had a diagnosis of probable AD according to NINCDS-ADRDA criteria (McKhann et al., 1984) and ICD-10 criteria. Patients were required to have Mini-Mental State Examination (MMSE) (Folstein et al., 1975) scores between 14 and 24 (both inclusive), and Modified Ischemia Scale (Rosen et al., 1980) scores of less than five. Exclusionary criteria were designed to ensure that patients had AD as the cause of their dementia. Patients with major depression, bipolar disorder, schizophrenia or schizoaffective disorder, transient organic psychotic disorder or psychoactive substance dependance were excluded. Computed tomography or magnetic resonance imaging within the past 12 months was required to exclude patients with cerebrovascular disease, hydrocephalus or intracranial mass lesions. Thyroid hormone levels and syphilis serology were evaluated to exclude patients with non-Alzheimer dementia. Furthermore, patients with a history of brain trauma, other neurological disease or polyneuropathy were excluded. Patients with significant illnesses (uncontrolled hypertension or hypotension, tachycardia or bradycardia, history of cancer, poorly controlled diabetes, history of heart failure, cardiac arrest, atrial fibrillation, recent myocardial infarction, significant pulmonary disorder) were also excluded. Concomittant use of psychoactive substances (antiepileptics, anticholinergics, antidepressants, monoamine oxidase inhibitors, neuroleptics, cognition enhancers, long-acting benzodiazepines), nootropic drugs, drugs with dilatory effects on cerebral blood vessels, or any investigational drug within 30 days prior to baseline examination was prohibited. Patients were allowed to take concomittant cardiac pharmacotherapy, antihypertensives, anti-inflammatories, antacids, as well as treat-ment for other concomittant metabolic disorders if these medications were taken at a constant dose since at least 30 days prior to the start of the study. In addition, low-dose neuroleptics, short acting benzodiazepines and antidepressants for low level sedation and sleep induction were allowed.

The study was conducted according to ICH-GCP guidelines and ethics comitee approval was obtained for each study centre. Written informed consent was given by the patient and his or her caregiver or legal representative.

Treatment regimen and patient allocation
Ready-to-use study medication was provided to the investigational sites by EBEWE Pharmaceuticals Ltd (Unterach, Austria). The active medication contained 30 ml Cere mixed with 70 ml of normal saline. Placebo was composed of 100 ml normal saline. Both the active drug and placebo were packaged in brown infusion bottles and were indistinguishable in appearance. The study medication was infused i.v. over a period of 20 min and infusions were administered daily on five consecutive days per week for 4 weeks. After a treatment-free interval of 8 weeks, this regimen was repeated, making up a total of 40 infusions per patient. The intermittent dosage regimen was based on the results of earlier studies, which demonstrated a clear benefit after 1 month of treatment, and that the effects are at least partly maintained beyond the cessation of the treatment. Since long-term daily i.v. application is not feasable, the regimen with intermittent dosing has been chosen.

Patients who met all entry criteria were assigned to the treatment groups in a 1:1 ratio, according to a randomization code generated by a computer (IFNS Ltd, Koeln, Germany). The randomization was carried out in blocks of six patients, stratified by study centre. The investigators and all other study personnel were blind as to the random code assignment until the completion of the statistical analysis.

Efficacy measures
Efficacy was evaluated based on the cognitive performance and the clinical global assessment of the patients. Primary efficacy measures were the Alzheimer's Disease Assessment Scale – cognitive subpart (ADAS-cog) (Rosen et al., 1984) and the Clinical Global Impressions (CGI) (Guy, 1976; CIPS, 1986). The CGI is a seven-point ordinal scale and our version provided for ratings from 1 to 8, where 5 reflected no change from baseline, ratings of 4, 3, 2, reflected increasing degrees of improvement of global impression and 6, 7 and 8 reflected increasing

worsening from baseline. A rating of 1 was used if the patient could not be assessed. Secondary outcome measures included the Syndrome-Short-Test (SKT) (Erzigkeit, 1989), the Montgomery-Asberg Depression Rating Scale (MADR-S) (Montgomery, 1979), the activities of daily living subpart of the Nuremberg Age Inventory (NAI) (Oswald, 1980), and the behavioural subpart of the ADAS, the ADAS-noncog (Rosen *et al.*, 1984).

Data from the primary outcome measures at week 16 was used for the primary analysis. The ADAS-cog and the CGI results were analysed using the mean score change from baseline and the corresponding responder rates. For the ADAS-cog, responders were defined as having an improvement of ≥ 4 points relative to baseline. CGI responders were defined as having a score < 5 (2, 3 or 4) which reflects patients who experienced improvement from baseline in clinical global impression. Patients who had no change (score 5) or even worsened (score 6, 7 or 8) were considered non-responders. The secondary analysis included the total score changes of the secondary efficacy parameters at weeks 4, 12, 16 and 28 relative to baseline and the analysis of the primary parameters at week 4, 12 and 28.

Safety measures

Treatment emergent adverse events (AEs), emergence of abnormal laboratory findings, changes in clinical laboratory tests, as well as changes in vital signs and general physical and neurological examinations were evaluated to determine the safety of Cere. AEs were rated by the investigators as mild, moderate or severe. The relationship to the study drug was rated using one of the following categories: none, unlikely, possible, probable or definite. These ratings were performed under blinded conditions.

Statistical analysis

The target sample size for this study was 144 randomized patients and was selected based on the CGI study results of earlier trials. The efficacy analyses in this study were performed on two patient populations: the Intent-to-Treat analysis (ITT) and the Per Protocol analysis (PP). The ITT analysis included all subjects who were randomized, received at least one dose of study medication and provided baseline data and a minimum of one post-baseline data point. In case of missing data, the last-observation-carried-forward method was applied. The PP analysis was defined as all patients who were randomized, fulfilled the study entry criteria, and received at least 80% of the infusions (16 infusions) during each treatment course. Furthermore, a subgroup analysis

was performed in a population of patients with a baseline MMSE score below 20.

Comparability of the two groups with regard to demographic and background characteristics was assessed using descriptive statistics and appropriate parametric and non-parametric statistical tests.

Efficacy variables were analysed using one-tailed statistics. $P \leq 0.025$ was considered statistically significant. Comparisons between treatment groups of the primary parameters ADAS-cog and CGI scores were made with a confirmatory statistical approach using the Wilcoxon test for independent samples. Group differences of the corresponding responder rates were analysed with Fisher's exact test. For the exploratory analyses of the secondary variables (ADAS-noncog, MADR-S, NAI, SKT), the Wilcoxon test for independent samples was used to compare the total score change from baseline and Fisher's exact test was used for the comparison of responder rates between treatment groups. Exploratory analysis of study centre differences was performed using the Kruskal–Wallis test, but revealed no significant result.

Safety analysis was carried out by comparing treatment groups with respect to incidence rates of AEs, laboratory abnormalities and neurological and physical examination findings. Changes from baseline in vital signs, laboratory tests and weight were also assessed and compared between the two groups. AEs were coded using the WHO adverse reaction terminology. Summary tables, presenting AEs by body system and preferred term were prepared for each group.

All statistical analyses were performed using the software programs SAS (SAS Institute Inc., Cary, NC, USA) and SPSS (SPSS, Chicago, IL, USA). No adjustments for multiple comparisons were made, since a significant improvement in both primary parameters was required to claim a positive outcome for the study.

RESULTS

Patient disposition

One hundred and forty-nine patients were randomized into two treatment groups: 76 patients to Cere and 73 patients to placebo. Of these patients, 76 of Cere and 71 of the placebo group received study medication and 70 and 66 completed the study, respectively. Reasons for study discontinuation are shown in Fig. 1. A total of 144 patients (74 Cere and 70 placebo patients) were included in the ITT population. Patients excluded from the analysis either received no treatment ($n = 2$) or had no post-base-
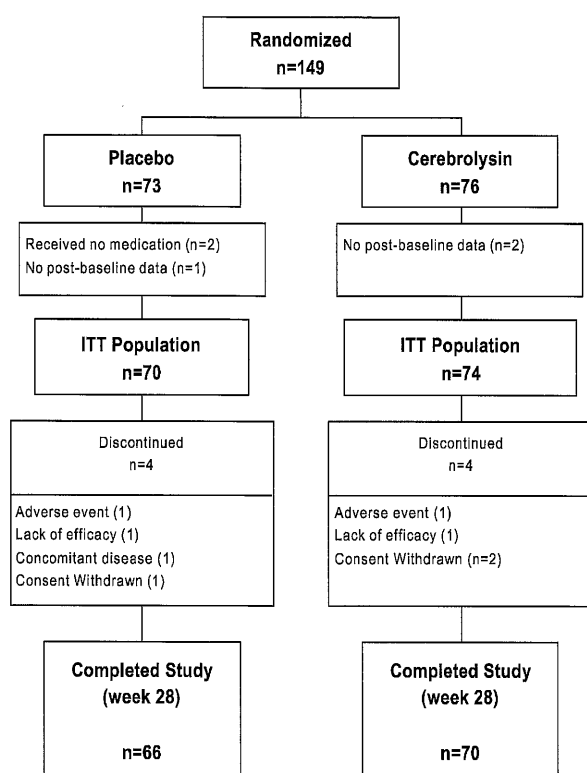
Randomized
n=149

Placebo
n=73

Cerebrolysin
n=76

Received no medication (n=2)
No post-baseline data (n=1)

No post-baseline data (n=2)

ITT Population
n=70

ITT Population
n=74

Discontinued
n=4

Discontinued
n=4

Adverse event (1)
Lack of efficacy (1)
Concomitant disease (1)
Consent Withdrawn (1)

Adverse event (1)
Lack of efficacy (1)
Consent Withdrawn (n=2)

Completed Study
(week 28)

n=66

Completed Study
(week 28)

n=70

Figure 1. Enrolment and disposition of patients participating in the clinical trial.

percentage of male patients was lower in the Cere group, but did not differ significantly from the placebo group ($P = 0.1283$).

The use of concomittant medications was homogeneously distributed between the two groups. Most frequently used medications were anti-inflammatories (Cere 44.6% versus placebo 47.1%), cardiac therapy (27% versus 31.4%), diuretics (18.9% versus 18.6%), mineral supplements (20.3% versus 10%) and calcium channel blockers (18.9% versus 11.4%). Nine patients (12.2%) of the Cere group compared to 10 patients (14.3%) of the placebo group reported the concomittant use of psychoactive substances (benzodiazepines, low dose neuroleptics and low dose antidepressants). There was no obvious difference between the two groups as regards the type of concomittant medications used nor as regards the frequency of concomittant drug intake.

The severity of disease at study entry and the global deterioration staging (GDS) were comparable between the two groups. Also, the baseline scores of the cognitive measures, the MMSE (Cere 17.0; placebo 17.5) and the ADAS-cog (Cere 32.0; placebo 30.2), showed no significant differences and were in accordance with a population of mild to moderate AD patients.

line data ($n = 3$). The PP dataset consisted of 137 patients (71 Cere and 66 placebo).

Demographic data and baseline disease characteristics

Baseline demographic and clinical characteristics of the patients are presented in Table 1. No significant group differences were observed at baseline. The

Efficacy

Table 2 summarizes the descriptive statistics for the primary and for selected secondary efficacy outcome measures for the ITT population. Mean change from baseline, standard error of mean, 95% confidence interval (CI) and exact propability are shown.

Cere-treated patients exhibited significantly superior clinical global impression when compared to

Table 1. Selected demographic data and baseline disease characteristics

	Treatment	
	Cerebrolysin ($n = 74$)	Placebo ($n = 70$)
Age (years)[a]	72.5 ± 0.92	73.5 ± 0.91
Gender (%)		
Male	26 (35.1)	34 (48.6)
Female	48 (64.9)	36 (51.4)
GDS (%)		
Stage 2	2 (2.7)	5 (7.1)
Stage 3	14 (18.9)	12 (17.1)
Stage 4	29 (39.2)	26 (37.1)
Stage 5	25 (33.8)	22 (31.4)
Stage 6	4 (5.4)	4 (5.7)
CGI – Severity of disease[a]	5.24 ± 0.07	5.16 ± 0.07
HIS[a]	3.0 ± 0.17	3.2 ± 0.16
MMSE[a]	17.0 ± 0.45	17.5 ± 0.53
ADAS-cog[a]	32.0 ± 1.44	30.2 ± 1.57

[a]Values are means ± SEM. No significant group differences were observed at baseline.

Table 2. Changes from baseline of primary and selected secondary efficacy parameters (ITT-analysis)

	CGI		ADAS-cog	
	Cere ($n = 74$)	Placebo ($n = 70$)	Cere ($n = 74$)	Placebo ($n = 70$)
Endpoint: Week 16				
Mean change from baseline	4.18 ± 0.11	4.60 ± 0.11	-2.1 ± 0.69	1.1 ± 0.59
Drug–placebo difference	-0.42		-3.2	
95% Confidence interval	$-0.12/-0.72$		$-1.42/-4.98$	
P (Cere versus placebo)	0.004		0.001	
Mean change at week 4	4.47 ± 0.08†	4.70 ± 0.10	-2.4 ± 0.49**	-0.4 ± 0.56
Mean change at week 28	4.81 ± 0.12	4.86 ± 0.12	0.0 ± 0.65*	1.6 ± 0.59

	NAI		ADAS-noncog	
	Cere ($n = 74$)	Placebo ($n = 70$)	Cere ($n = 74$)	Placebo ($n = 70$)
Endpoint: Week 16				
Mean change from baseline	-0.5 ± 0.29	0.0 ± 0.21	-1.2 ± 0.45	-0.2 ± 0.29
Drug-placebo difference	-0.5		-1.0	
95% Confidence interval	$-1.2/0.2$		$-2.05/0.05$	
P (Cere versus placebo)	0.071†		0.003	
Mean change at week 4	-0.3 ± 0.25	-0.3 ± 0.19	-1.3 ± 0.24**	-0.3 ± 0.25
Mean change at week 28	0.0 ± 0.32†	0.4 ± 0.30	-0.1 ± 0.38*	0.9 ± 0.37

Values are means $\pm$ SEM; For the CGI, lower scores indicate improvement. For the ADAS-cog, the NAI and the ADAS-noncog, negative score changes indicate improvement. †$P < 0.1$; *$P < 0.025$; **$P < 0.01$.

placebo-treated patients at the week 16 primary endpoint of the study, after the end of the active therapy. The mean treatment difference was 0.42 points on the CGI ($P = 0.004$) in favour of Cere. A responder analysis (CGI score < 5) showed that 63.5% of Cere-treated patients compared to only 41.4% of placebo-treated patients responded to therapy ($P = 0.006$). These results were confirmed in the PP dataset, with a mean treatment difference in favour of Cere of 0.47 points ($P = 0.023$) and a responder rate of 64.8% for Cere versus 40.9% for placebo ($P = 0.004$).

At week 28, according to the responder analysis, patients treated with Cere still had superior CGI scores compared to placebo patients. At this visit, 45.9% of the Cere-treated patients had improvement of the CGI from baseline, in contrast to only 28.6% of the placebo-treated patients ($P = 0.024$). This indicates that the beneficial effect of Cere was at least partially maintained for up to 3 months after drug withdrawal.

Comparable results were observed in the cognitive domain, the second primary parameter of this study. On the ADAS-cog, a significant treatment effect of Cere was observed at the week 16 visit, with 47.3% of Cere patients compared to 15.7% of the placebo patients having improved by 4 or more points from their baseline score ($P < 0.001$). Patients on Cere had a mean improvement from baseline of -2.1 points on the ADAS-cog, while placebo patients worsened by 1.1 points, thus accounting for a treat-

ment difference of -3.2 points (CI $-1.42/-4.98$; $P < 0.001$) in favour of Cere. These results were confirmed in the PP population (group difference -3.2 points; CI $-1.34/-5.06$; $P < 0.001$).

Changes in the ADAS-cog over time are depicted in Fig. 2. The Cere group showed a marked treatment benefit after the first treatment cycle, an improvement in the ADAS-cog of -2.4 points. Minor improvement was also observed in the placebo group at the week 4 visit (-0.4 points), but placebo patients started to deteriorate immediatly thereafter, whereas the patients on Cere had a sustained treatment effect up to the week 16 visit, after the second treatment cycle. Subsequently, the patients on Cere also started to lose some of the treatment benefit. Remarkably, however, at the week 28 visit, there was still a significant group difference in favour of Cere ($P = 0.016$). Placebo patients had deteriorated by 1.6 points in the ADAS-cog by that time, while patients on Cere had maintained their baseline cognitive performance levels. Again, essentially similar results were observed in the PP population, with a treatment difference of -1.5 points in favour of Cere ($P = 0.024$).

Improvement of cognitive function correlated well with improvement of the clinical global score. At week 16, 27 of the 47 Cere-treated patients (57.4%) who responded in the CGI also showed an improvement of ≥ 4 points in the ADAS-cog. This fraction was much lower in the placebo group, with 27.5% of CGI responders also meeting the response criteria
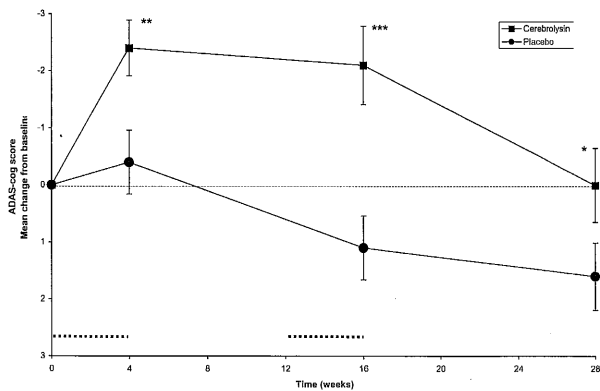
Figure 2. Time course of the ADAS-cog: mean change from baseline ($\pm$SEM) of Cere-treated and placebo-treated patients. ITT analysis, $n = 74$ for Cere and $n = 70$ for placebo. Negative score differences indicate improvement. $^{*}P < 0.025$, $^{**}P < 0.01$, $^{***}P < 0.001$. Dashed lines indicate the timing of the infusion treatment.

Figure 4. Activities of Daily Living (NAI score): mean change from baseline ($\pm$SEM) of Cere-treated and placebo-treated patients. ITT analysis, $n = 74$ for Cere and $n = 70$ for placebo. Negative score differences indicate improvement. $\dagger P = 0.071$. Dashed lines indicate the timing of the infusion treatment.

in the ADAS-cog. The percentage of combined responders was significantly higher in the Cere group, at week 16 as well as at week 28 (Fig. 3).

The results of the secondary outcome parameters provided supportive evidence for the efficacy of Cere, most prominently in the activities of daily living and behavioural disturbances.

In the activities of daily living (NAI score) treatment differences at the study endpoint favoured the Cere group. Although not reaching statistical significance, there was a clear trend ($P = 0.071$) in favour of Cere with a drug-placebo difference of 0.5
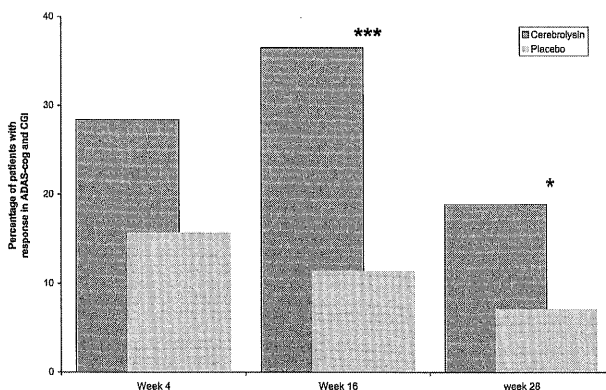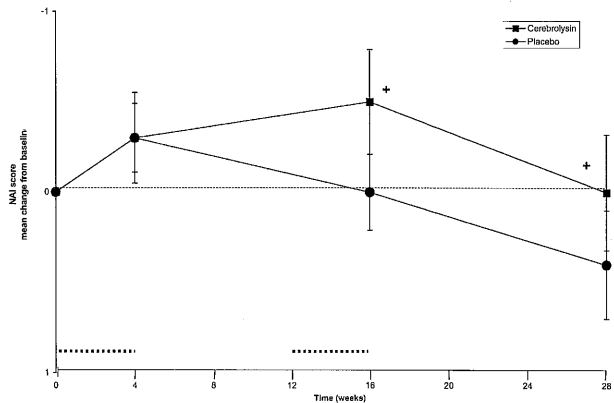

Figure 3. Responder analysis. Percentage of patients with treatment response in both primary outcome measures, in the CGI (score < 5) and in the ADAS-cog (improvement from baseline ≥ 4 points). ITT analysis, $n = 74$ for Cere and $n = 70$ for placebo. $^{*}P < 0.025$, $^{***}P < 0.001$.

points (CI $-1.2/0.2$). Improvement of activities of daily living was evident after 1 month in both groups. Patients on placebo started to deteriorate thereafter, returning to baseline levels at week 16 and finally worsening by 0.4 points in the NAI at the end of the study. In contrast, in the Cere group, the improvement seen after 1 month was maintained and the patients even experienced further benefit up to -0.5 points at the week 16 visit. Cere patients then started to deteriorate slowly in the washout phase, from week 16 to the week 28 visit, at which time they got back to their baseline levels, but still performed 0.4 points better than the control group ($P = 0.071$) (Fig. 4).

A significant superiority of Cere over placebo was evident in the ADAS-noncog (Table 2). The treatment difference at week 16 was -1.0 points in favour of Cere ($P = 0.003$). This favourable effect was fully maintained until after the washout phase, at which time patients on Cere had improved by -0.1 point, whereas placebo treated patients had deteriorated by 0.9 points.

The analysis of the remaining secondary efficacy measures (MADR-S, SKT) revealed no important effects. Both treatment groups showed a minor improvement from baseline at week 4 and week 16 in the ITT and the PP populations, and patients of both groups were back to their baseline scores at week 28. The improvement was greater for the Cere-treated patients, and at week 28 the difference between the two groups favoured Cere, but did not reach statistical significance ($P = 0.161$ for SKT; $P = 0.102$ for MADR-S).

Table 3. Results of subgroup analysis of patients with MMSE score < 20 at baseline

	Week 16 Visit		Week 28 Visit	
	Cere ($n = 56$)	Placebo ($n = 44$)	Cere ($n = 56$)	Placebo ($n = 44$)
CGI				
Score[a]	4.18 ± 0.13	4.91 ± 0.13	4.80 ± 0.14	5.11 ± 0.13
Drug/Placebo diff.	−0.73***		−0.31†	
ADAS-cog				
Score change[a]	−2.8 ± 0.82	1.5 ± 0.78	−0.7 ± 0.72	2.2 ± 0.84
Drug/placebo diff.	−4.3***		−2.9**	
NAI				
Score change[a]	−0.4 ± 0.33	0.2 ± 0.29	0.0 ± 0.37	0.6 ± 0.41
Drug/Placebo diff.	−0.6†		−0.6	
ADAS-noncog				
Score change[a]	−1.0 ± 0.54	0.3 ± 0.39	0.0 ± 0.39	1.7 ± 0.51
Drug/Placebo diff.	−1.3***		−1.7***	

[a]Values are means ± SEM. Negative differences represent improvement. †$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Subgroup analysis

To explore the effects in patients with moderate AD a subgroup analysis of patients with MMSE scores < 20 at baseline was performed. One-hundred subjects, 56 Cere and 44 placebo, were included in this analysis. The findings of the ITT analysis were confirmed in this subgroup but drug-placebo differences were even more pronounced. This was largely due to a markedly reduced response of placebo patients in this sample, whereas the response of patients to Cere either remained unchanged or was slightly higher when compared to the ITT sample. In this subgroup, again, a significant superiority of Cere over placebo was evident for both primary parameters at the study endpoint (Table 3). In addition, borderline statistically significant results were observed in the activities of daily living (NAI). At week 16, patients on Cere improved by 0.4 points and placebo patients deteriorated by 0.2 points, accounting for a drug-placebo difference of 0.6 points in favour of Cere ($P = 0.0251$).

Safety

One hundred and forty-seven patients constituted the safety population with 76 patients receiving Cere and 71 receiving placebo. Two of 149 randomized patients received no treatment and thus were excluded from the safety analysis.

The overall incidence of AEs was similar in both treatment groups, 43.4% of Cere patients compared to 38.0% of placebo patients experienced at least one AE. Four patients reported serious adverse events (SAE), two in each group. In all four cases, there was no causal relationship to the study drug, as per the investigator's assessment. No deaths occurred during this study.

Most AEs were rated mild in both treatment groups. Events rated severe were reported with a

Table 4. Patients with adverse events (%)

	Treatment	
Adverse event[a]	Cerebrolysin ($n = 74$)	Placebo ($n = 70$)
Any adverse event	33 (43.4)	27 (38.0)
Mild	29 (38.2)	25 (35.2)
Moderate	22 (28.9)	24 (33.8)
Severe	13 (17.1)	13 (18.3)
Vertigo	25 (32.9)	20 (28.2)
Headache	19 (25.0)	16 (22.5)
Increased sweating	18 (23.7)	16 (22.5)
Nausea	16 (21.1)	17 (23.9)
Tachycardia	4 (5.3)	4 (5.6)
Bronchitis	3 (3.9)	4 (5.6)
Flu symptoms	3 (3.9)	4 (5.6)
Rhinitis	4 (5.3)	1 (1.4)

[a]Adverse events are encoded by WHO Adverse Reaction Terminology. Only adverse events with an incidence rate of > 5% in one of the two treatment groups are presented.

similar frequency in both groups (Cere 17.1%; placebo 18.3%). AEs with an incidence rate of at least 5% in one of the two treatment groups are presented in Table 4. The most common AEs reported by patients on Cere were vertigo, headache, increased sweating and nausea. These AEs, however, occurred with a similar frequency in the placebo group (Table 4).

No clinically important difference between the two treatment groups for any laboratory tests including haematology, blood chemistry and urinalysis was observed. Furthermore, Cere produced no clinically significant changes in the vital signs. Overall, Cere was well tolerated and no systemic pattern of toxicity for Cere was observed.

DISCUSSION

This study confirms that treatment with i.v. infusions of Cere results in statistically and clinically significant improvement of CGI and cognitive performance in patients with mild to moderate AD. Beneficial effects were also seen in the behavioural domain (ADAS-noncog) and the activities of daily living (NAI). This is an important observation, since the behavioural problems and loss of independence of the patient are the most distressing factors associated with AD. They are largely responsible for the severly impaired quality of life of both the patient and the caregiver.

The positive findings of this study are robust. For all study populations analysed (ITT, PP and subgroup analysis), a significant treatment benefit for Cere was observed. The difference in numbers of responders on the CGI was 22% in the ITT population, 24% in the PP-population and 29% in the subgroup of patients with MMSE scores of less than 20. The percentage of placebo responders in the global score at the week 16 visit appeared to be relatively high compared to other studies in AD. Although the exact reason for that remains elusive, we believe that the extra amount of care associated with the daily interaction with the nurse administering the i.v. therapy may have contributed to the above average placebo response. The mean CGI score difference between the two groups was 0.42 points, and is among the largest reported to date. These results are in accordance with the findings of earlier trials with Cere (Ruether et al., 1994; Panisset et al., 2000) where significant improvement in the global rating were reported.

A good correlation of CGI and ADAS-cog response was observed in this study. Thus, the im-provement of the global score was probably predominantly driven by the concomittant highly significant improvement of cognitive performance. Other factors which may have contributed to the beneficial effect on the clinical global impressions include the amelioration of behavioural disturbances (ADAS-noncog) and the improved activities of daily living (NAI) of the patients in the Cere group.

Estimates of the rate of cognitive decline over time have ranged up to 9 points on the ADAS-cog in 1 year (Stern et al., 1994). The stabilization of cognitive decline seen over 28 weeks in this trial is therefore of considerable relevance for the clinical practice. Similarly, although the overall benefit in the CGI with a group difference of 0.42 points may seem small, no decline of the mean clinical global impressions score from baseline was seen over 7 months, with 45.9% of Cere patients even having improved from their baseline condition. Given the expected natural deterioration of people suffering from AD, this observation is also highly clinically relevant to the patient and the carer.

In spite of the enormous efforts in AD research, only symptomatic treatments are currently available. Modern cholinesterase inhibitors, such as donepezil (Doody, 1999) or rivastigmin (Anand et al., 1996) are well tolerated and safe, but provide only symptomatic benefit for a limited time. The clinical benefit of cholinergic treatment for AD in improving cognitive function, expressed as the drug/placebo difference on the ADAS-cog has ranged from 1.6–2.57 points. We have observed a similar magnitude of benefit with a difference of 3.2 points. Similarly, the treatment difference to placebo of 0.42 points on the global score is comparable to what has been demonstrated for cholinesterase inhibitors, with drug/placebo differences ranging from 0.28–0.47 points (Morris et al., 1998; Rogers et al., 1998; Roesler et al., 1999).

With cholinergic agents, this treatment effect vanishes immediately after drug withdrawal. Rogers et al. (1998) reported that the effect of donepezil was lost within 6 weeks after cessation of 26 weeks of continuous treatment. Cere, however, exhibits quite different properties from cholinesterase inhibitors. Ruether et al. (2000) reported a persisting efficacy of Cere over a period of 6 months after cessation of active treatment. Panisset et al. (2000) detected a signifcant treatment effect for Cere 2 months after the treatment was stopped. Similarly, in this study, the positive effects on global function, cognitive performance, behavioural disturbances and activities of daily living observed at the end of the active treatment phase were largely maintained 3 months

after drug withdrawal. Although the beneficial effect started to vanish slowely after cessation of the treatment, statistically significant superiority of Cere over placebo was still evident at the week 28 visit. These data may indicate a possible disease modifying action and stabilizing effect of Cere which goes far beyond a pure symptomatic effect.

This long-term effect is in agreement with the purported neurotrophic activity of Cere. Experimental studies with Cere in rats have consistently shown effects on brain histology and behaviour. After 1 month of treatment, persisting long-term improvements were observed (Gschanes and Windisch, 1998; Valouskova *et al.*, 1998; Masliah *et al.*, 1999). Cere led to an increased density of dentritic arborization and to increased synaptic density in these animals (Masliah *et al.*, 1999; Reinprecht *et al.*, 1999). It can be expected that such profound effects on neuronal cytoarchitecture are capable of inducing long-term clinical benefits.

This clinical trial confirms the safety and tolerability findings of earlier clinical trials with Cere, which have demonstrated an excellent safety profile for this compound. Panisset *et al.* (2000) reported adverse events with a similar frequency for Cere and placebo treatment. In this study also, adverse events occurred with a similar frequency in both groups. Furthermore, no changes on laboratory parameters or vital signs were noted. In both groups, the most common adverse events were vertigo, headache and sweating. These events represent unspecific symptoms, which can probably be attributed to the infusion procedure per se, rather than to the study medication. Overall, Cere was well tolerated by the patients and no systemic pattern of toxicity was observed for Cere.

In conclusion, the neurotrophic compound Cerebrolyisn is safe and effective for the treatment of patients with AD and leads to a statistically significant and clinically relevant improvement of cognitive performance and clinical global impressions. Most importantly, the therapeutic benefit is maintained in part for at least 3 months after drug withdrawal, suggesting a stabilizing effect of Cere in patients with AD. Long-term studies are warranted to further explore the possibility for Cere to slow the progression of AD. Issues such as the optimal therapy-free interval between successive treatments will need to be addressed.

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