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W.-D. Heiss
J. Kessler
R. Mielke
B. Szelies
K. Herholz

Max-Planck-Institut für neurologische Forschung und Neurologische Universitätsklinik Köln, BRD

Long-Term Effects of Phosphatidylserine, Pyritinol, and Cognitive Training in Alzheimer's Disease

A Neuropsychological, EEG, and PET Investigation

Key Words
Alzheimer's disease
Phosphatidylserine
Cognitive training
Positron emission tomography
EEG
Neuropsychological evaluation

Abstract
70 patients with probable Alzheimer's disease were randomly allocated to four groups: 17 patients received only social support, 18 cognitive training twice a week, in 17 cognitive training was combined with pyritinol 2 × 600 mg/day and in 18 cognitive training was combined with phosphatidylserine 2 × 200 mg/day. Treatment duration was 6 months. Before and after treatment, the patients underwent neuropsychological testing as well as measurement of the regional cerebral metabolic rate for glucose using positron emission tomography and ^{18}F-2-fluoro-2-deoxy-*D*-glucose. Before treatment the groups were comparable in respect to resting and activated glucose pattern achieved by a visual recognition task. Electrophysiological changes were assessed as EEG power, globally and in 4 frequency bands. This 6-month study in four groups of patients with Alzheimer's disease indicated that phosphatidylserine treatment has an effect on different measures of brain function. Since neuropsychological improvements were best documented after 8 and 16 weeks and faded towards the end of the treatment period, it must be concluded that this symptomatic therapy is mainly of short-term benefit and was overcome by the progressive pathological changes at the end of the treatment period.

Introduction

Alzheimer's disease (AD), the most common type of primary degenerative dementia and the leading cause of cognitive impairment in the elderly, is due to molecular changes of proteins of the nerve cell's membrane or cytoskeleton and therefore up to now not amenable for causative treatment. As long as strategies aimed at repairing, reducing or delaying the degenerative process in the neurons are not available for clinical application, symptomatic treatment approaches must be adopted by which the cognitive deficits can be alleviated and the progression of memory and neurologic impairment retarded. Since the loss of cholinergic neurons and the decrease of choline acetyltransferase [1, 2] correlate best with the degree of memory impairment [3], therapeutic trials have concentrated on enhancing cholinergic activity [4] with some effects on cognitive and memory reported for cholinesterase inhibitors [5, 6] as well as cholinergic agonists [7]. However, in addition to the cholinergic deficits, other

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W.-D. Heiss, MD
MPI für neurologische Forschung
Gleueler Strasse 50
D-50931 Köln-Lindenthal (FRG)

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transmitter systems are involved in AD [8] and additional disturbances of metabolism and membrane function contribute to the progression of functional and morphologic damage in AD. Therefore, therapeutic strategies targeted against multiple transmitter systems lesions, protecting against toxic effects of excitatory amino acids and increased Ca^{2+} influx, improving disturbed cell metabolism, and stabilizing membranes may be effective in ameliorating cognitive deficits in AD [4, 9–11]. Additionally, with the importance of regular training of memory documented for preservation of optimal function in normal aging [12], attempts were also made to improve memory and cognitive function in AD, but the effects were controversial [13].

The evaluation of treatment effects in AD is a tedious task necessitating large numbers of patients and long observation periods [5–7]. To keep efforts and costs within acceptable limits, only those drugs that have been shown to possess some potential for therapeutic efficacy can be entered into large-scale clinical trials. Based on the mode of action demonstrated in animal experiments, the rationale for a drug's effect can be tested in selective clinical studies in which a quantifiable parameter related to the course and severity of the disease is used as the target variable. With this approach of a targeted investigation, intermediate between experimental studies and controlled clinical trials, expensive large-scale clinical trials can be concentrated on those candidates shown to be effective on a quantified marker of the disease. As impairment of memory and other cognitive disturbances can be related to decreased glucose metabolism in brain regions predominantly affected by AD, improvement in glucose utilizations may ameliorate cognitive decline. Therefore, studies of regional cerebral metabolic rate of glucose ($rCMR_{glu}$) by positron emission tomography (PET) may be of value in the preclinical evaluation of drug therapy in this degenerative disorder.

Phosphatidylserine (PS), the major acidic phospholipid in the brain, which also penetrates into the brain after oral application [14], has been shown to affect multiple neurochemical systems, the neuronal membranes [15] as well as cell metabolism [16], and specific neurotransmitter systems including acetylcholine [17], norepinephrine [18], serotonin [19] and dopamine [20]. Clinical studies have indicated improved cognitive function in relation to patients under placebo [21, 22].

Nootropic substances and enhancers of brain activity such as pyritinol [23, 24] are often used for symptomatic treatment of AD, and they may be effective by their activation of brain metabolism. In analogy to the positive influence of memory training on forgetfulness in higher age [25], cognitive training programs may also be utilized in AD, and their efficacy might be enhanced by drugs stabilizing neuronal metabolism [26]. Therefore, a controlled parallel group pilot study was designed in which the effectiveness of PS plus cognitive training was compared with cognitive training alone, cognitive training plus pyritinol, and with social support alone. In order to evaluate various dimensions of efficacy, $rCMR_{glu}$ measurements, quantitative electroencephalogram (EEG) and neuropsychological tests scores were obtained before and after a 6-month treatment period.

Methods

Patients

Eighty outpatients of both sexes, between 48 and 79 years, who met the NINCDS-ADRDA [27] criteria for AD, were enrolled in the study. The patients were recruited from a total of 350 patients seen in the Memory Clinic running on a special grant from BMFT (Federal Department of Science and Technology) at the Department of Neurology of the University of Cologne. All patients were mildly to moderately demented, scoring between 13 and 26 on the Mini-Mental State Examination (MMSE) [28], were living in the community and required no concurrent medication known to affect the central nervous system. All patients had a regular caregiver who was available throughout the study to monitor medication and to provide information about patient status. Informed consent was obtained from the patient and the caregiver, usually the next of kin. The study was approved by the Ethics Committee of the University of Cologne Medical School.

Procedures

Patients were admitted to the Department of Neurology of the Cologne University Hospital for baseline assessments. These consisted of a general medical and detailed neurologic and psychiatric examination, a lumbar puncture, complete blood cell counts, routine blood chemistry determinations, thyroid function test, serum vitamin B_{12} level measurements and a battery of neuropsychological tests. In addition, ultrasound examination of the neck vessels and X-ray computed tomography or/and magnetic resonance imaging were employed to exclude multi-infarct dementia, brain tumors, and other organic brain diseases that could have caused secondary dementia. Additionally, the modified Hachinski score of Rosen et al. [29] was used; only patients with a score of 3 or less were included in the study. Their EEG was recorded and analyzed quantitatively and their $rCMR_{glu}$ was studied with PET at rest and during activation by a stimulation task. Patients were then randomly assigned to the four groups, but blinding was not attempted since the strategies were completely different and the tedious blinding procedures seemed unjustified in this exploratory pilot study.

After discharging the patients the 4 respective regimens were followed: (1) Social support (SS): During 1 h once a week the patients spoke about their personal problems and how they managed their daily life. Experiences from their past took up a great deal of time. Sometimes games were used to support the conversations. (2) Cogni-

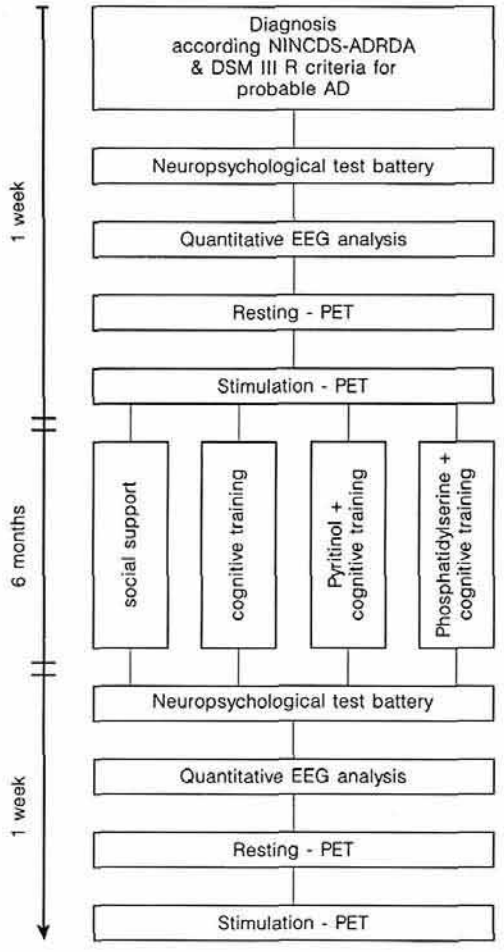


Fig. 1. Scheme of the study protocol.

tive training (CT): A Commodore C64 computer with a color monitor was used for cognitive training. For 1 h twice a week, the patients had to solve different memory, perceptual or motor tasks, which varied in the degree of difficulty. The tasks were selected regarding the patients' profile of cognitive impairment. Only programs of 'Rigling Reha-Service' [30] were used. (3) CT as in group 2 in combination with oral pyritinol 600 mg twice daily (CT-P). (4) CT as in group 2 in combination with oral phosphatidylserine 200 mg twice daily (CT-PS).

All patients and their caregivers were seen regularly by members of the team at their visits to receive social support or training, and at 4-week intervals by a physician, when they were asked about adverse effects and underwent a short neurologic/psychiatric examination and when a month's supply of medication was dispersed. The last supply of study medication was collected and counted to assess compliance. Complete blood cell counts and routine blood chemistry determinations were repeated at a 4-week interval during treatment duration. MMSE and several neuropsychological tests were repeated after 8 and 16 weeks, and all neuropsychological tests as well as EEG and PET of $rCMR_{glu}$ at rest and during activation were done after completion of the 6-month treatment period. The protocol is schematically shown in figure 1.

Outcome Measures

Three levels of outcome measures were assessed: performance in neuropsychological tests and MMSE, functional status of the brain assessed by EEG power, and $rCMR_{glu}$ at rest and during activation. Since a blinded study was not attempted, clinical impression and instruments for rating activities of daily living, mood and disturbance of behavior were not evaluated.

Neuropsychological Test Battery. The following tests were used before entry and after completion of the treatment period. The neuropsychological test battery is described more extensively elsewhere [31]. Severity of dementia was rated with the MMSE [28]. Memory was assessed by a verbal and pictorial selective reminding paradigm with a delayed recognition task [32]. Visuospatial short-term memory was tested by a Corsi's tapping task [33]. Perceptual memory and priming was recorded by a modification of Gollin's incomplete picture test [34]. Language capacity was assessed with a verbal fluency task [32] and a subtest of the token test [35]. For reaction time measurement a go/no go paradigm was used [36]. Concentration capacity was measured with a 'concentration test for elderly people' (Alters-Konzentrations-Test) [37]. Handedness and psychomotoric speed were assessed with a modified laterality questionnaire after Oldfield [38] and a tapping task with both hands. Finally, items for orientation and praxia were included. The whole test battery was used before and after therapy. After 8 and 16 weeks the items of the MMSE, the selective reminding task with delayed recognition, praxia and orientation were applied for documenting the time course.

Quantitative EEG Analysis. Within 24 h before or after PET, EEG was recorded using an online 19 channel EEG system (Brain Atlas III Biologic) with 16 scalp electrodes (F_3, F_4, C_3, C_4, C_Z, P_3, P_4, P_Z, O_1, O_2, F_7, F_8, T_3, T_4, T_5, T_6) according to the 10–20 system with linked mastoid reference (Silver-Grass electrodes, impedance less than 3 kΩ, gain 20,000, filter 1.0 and 30.0 Hz, sampling rate 128 Hz). Frequency analysis of at least 15 artefact-free 2-second periods and calculation of power spectra after fast Fourier transformation was performed. Global power and frequency band power was calculated summarizing in 0.5-Hz steps for delta (2–3.5 Hz), theta (4–7.5 Hz), alpha (8–13 Hz) and beta (13.5–20 Hz). Relative power was calculated by dividing absolute power of each band by total power.

Positron Emission Tomography. ^{18}F-2-fluoro-2-deoxy-*D*-glucose (FDG) was used to measure $rCMR_{glu}$ by PET [39]. Each subject was studied at rest and during stimulation usually on successive days before randomization and after completion of the treatment period. In the resting condition the patients were supine with eyes closed, ears unplugged, in a darkened room with low ambient noise. As a stimulation procedure, a continuous visual recognition task with different degrees of difficulty adjusted to each individuals performance capacity was used [31]. It consisted of 200 items, e.g. black and white pictures of concrete objects, numbers, words, nonsense syllables, combination of letters and numbers, figures from the recurring figure test of Kimura [40] and the random shapes of Vanderplas and Garvin [41]. Verbal and nonverbal items were balanced in order to obtain bihemispheric cortical stimulation. The degree of difficulty of the task to be used was determined for each subject by the results achieved in the neuropsychological test battery. Subjects were in-

structed to identify any previously presented picture among the new ones with spoken yes/no responses. Each picture was presented for at least 5 s, the intertrial interval was usually 3 s. The task was performed for 30 min in a sitting position and was started immediately after FDG injection. After completion of the task the patients were transferred to the scanner, and the measurement of brain FDG activity was performed in supine position.

Two different PET scanners were used in the study, but each patient had all studies on the same scanner. Most patients (n = 52) were recruited before August 1991 and had all examinations on a four-ring (7 slice) PET scanner (Scanditronix PC384, in-plane resolution 7.8 mm, 11 mm slice thickness). Brain activity was recorded in two table positions from 30 to 50 min after i.v. injection of 185 MBq FDG, resulting in a total of 14 transaxial slices. 18 patients were entered after August 1991 and had all examinations on a 24-ring (47 slice) scanner (Siemens CTI, ECAT EXACT, 5 mm transaxial, 6 mm axial resolution) [42] after i.v. injection of 370 MBq FDG. Arterialized venous blood samples were withdrawn at predetermined intervals as described previously [43], and used together with the measured tissue activity to calculate $rCMR_{glu}$ [44] with a fixed lumped constant of 0.42.

For the quantitative regional analysis of $rCMR_{glu}$, the first resting study of each patient was oriented in parallel to the anterior commissure-posterior commissure (ACPC) line by comparison of a mid-sagittal slice with a correctly positioned reference scan using an interactive procedure for three-dimensional alignment [45]. Subsequently, the other scans of each patient were aligned with the first resting scan. Data were then reformatted to a set of 14 contiguous slices with 6.85 mm slice thickness, ranging from 41 mm below to 48 mm above the ACPC line. Onto this data set, standardized regions derived from the atlas of Matsui and Hirano [46] and adapted to the individual brain shape were positioned by an interactive mapping program (modified from Herholz et al. [47]). Besides absolute $rCMR_{glu}$ values (in µmol glucose per 100 g tissue/min), relative values calculated by dividing the regional values through the average value of all regions in each patient were also used for statistical analysis to reduce interindividual variance.

Data Analysis

Data were generally described as mean values and standard deviations for each treatment group. Groups were generally compared by analysis of variance (ANOVA) with Tukey's multiple means comparison. To avoid bias due to possible nonnormal data distribution, significant differences among groups were confirmed by nonparametric Kruskal-Wallis tests. Longitudinal changes of parameters during the study were analyzed by Wilcoxon test with reference to the respective pretreatment value. All calculations were performed with the use of a commercial software package (SAS Institute, Cary, N.C.).

Results

A total of 80 patients entered the study, but complete data were available in 70 cases only with dropouts due to technical insufficiencies of PET, EEG data or side effects. Patient characteristics for the entire study population are summarized in table 1. The groups were comparable in education and occupation, based on attendance of intermediate schools and professional training. There were no differences in premorbid IQ estimation between the groups. No statistically significant differences at the metabolic, EEG and neuropsychological level were noted at the baseline between the population of the respective treatment groups. Changes over a period of 6 months were assessed with respect to 3 functional levels.

Table 1. Demographic variables

	Age, years	Sex	
		M	F
SS	66.63 ± 10.17	10	7
CT	65.95 ± 6.28	9	9
CT-P	67.18 ± 8.51	8	9
CT-PS	66.74 ± 6.93	10	8

Neuropsychological Results

Between Groups. The neuropsychological data are summarized in table 2. There was no significant difference in the four groups in severity of dementia expressed in MMSE scores or in premorbid IQ (premorbid IQ estimation [32]; the verbal intelligence test from Lehrl [48]). According to reference scores all groups were significantly impaired in memory, language, orientation, praxia and concentration tasks, but there were no differences between the groups. Scores in the orientation questionnaire were significantly higher in CT-PS group after 8 weeks treatment compared to the CT and SS groups ($p = 0.0049$), but not between the CT-PS and the CT-P group. During week 16 the CT-PS group was again significantly superior in orientation compared to groups SS and CT ($p = 0.0161$ resp. 0.0339). At the end of therapy after 6 months differences between groups were no longer significant.

Within-Group Comparison. SS group: no significant improvement in any test was observed; *CT group:* no significant improvement in any test was observed; *CT-P group:* patients had significantly higher orientation scores after 8 weeks ($p = 0.05$) and produced significantly more words in the verbal fluency task after 16 weeks; no other significant improvements were observed; *CT-PS group:* the patients had significantly higher MMSE scores after 8 weeks ($p = 0.0045$), after 16 weeks ($p = 0.05$), and showed a tendency of improvement after therapy with phosphatidylserine ($p = 0.08$). Orientation scores improved after 8 weeks ($p = 0.0001$), after 16 weeks ($p = 0.0027$), and again a tendency to improvement was shown in week 26. They

Table 2. Neuropsychological test battery

	Max. score	CT				CT-P			
		before	8 weeks	16 weeks	after	before	8 weeks	16 weeks	after
Degree of dementia									
MMSE	30	20.55±4.42	20.50±5.24	20.11±5.36	19.33±6.82	21.64±4.55	21.35±5.75	20.82±6.29	21.47±6.66
Memory									
Verbal selective reminding	8	3.62±1.30	3.56±1.53	3.65±1.64	4.03±1.62	3.93±1.65	4.08±1.35	4.18±1.48	4.12±1.61
Recognition (hits)	8	6.35±1.76	6.58±1.90	6.50±2.00	7.25±1.00	6.64±1.72	6.82±1.46	6.52±1.12	6.87±1.62
Recognition (false-pos.)	24	4.00±4.44	3.05±3.84	3.43±4.78	4.06±5.63	3.00±5.35	2.35±3.77	1.35±1.32	1.93±1.98
Corsi's blockspan	9	4.21±1.32	–	–	3.57±1.67	33.60±2.23	–	–	4.00±1.59
Fragmented picture test (differences)	50	5.43±3.36	–	–	4.07±7.85	6.40±4.77	–	–	6.12±4.01
1st present.	50	31.87±4.74	–	–	36.69±4.88	35.93±6.91	–	–	41.52±5.43
2nd present.	50	26.43±5.69	–	–	32.51±8.01	29.00±9.44	–	–	35.62±8.38
Language									
Supermarket		10.77±4.30	10.66±5.65	11.33±4.15	12.43±5.84	11.82±8.14	12.58±7.39	13.94±8.17	11.76±7.06
Token test	10 errors	3.06±2.61	–	–	2.46±2.56	3.33±3.28	–	–	3.82±3.94
Orientation, praxia									
Orientation	8	4.77±2.04	5.11±2.17	5.61±2.89	5.37±2.36	5.52±1.90	6.11±1.83	6.00±2.00	5.87±2.24
Praxia	10	7.22±2.60	7.38±2.35	7.05±2.75	6.81±2.99	7.76±2.51	7.94±2.41	7.47±2.76	6.52±3.00
Attention, concentration, reaction time									
Reaction time		0.66±0.12	0.72±0.13	0.69±0.12	0.67±0.12	0.68±0.13	0.69±0.14	0.64±0.14	0.68±0.14
AKT (t)		56.38±19.32	–	–	60.55±30.0	61.04±41.98	–	–	64.10±33.85
Tapping task		327.86±55.90	–	–	343.15±29.49	332.66±53.51	–	–	333.17±45.20

also reached a significant improvement in the visuospatial digit span after therapy ($p = 0.0038$).

Frequency of Responders and Nonresponders in Each Group. Each therapy group was divided into responders, as defined by improvement of 3 or more in the MMSE score at the end of therapy, and nonresponders. With this criterion, in the SS group 18% (n = 3), in the CT group 11% (n = 2), in the CT-P group 24% (n = 4), and in the CT-PS group 50% (n = 9) were responders. Fisher's exact test with a 4 × 2 contingency table showed significant group differences, whereby the CT-PS group had significantly more responders than the CT group ($p = 0.0137$) or the SS group ($p = 0.0474$). The rate of responders was not significantly different between groups CT-PS and CT-P.

Quantitative EEG Analysis

As demonstrated before [49], quantitative analysis of EEG power revealed changes of electrophysiological brain activity in AD. These changes, albeit with some regional differences which were especially pronounced in temporoparietal projection but without significant selectivity, were most conspicious in global analysis and consisted of a moderate decrease of global power with a marked increase of theta and less pronounced delta, and decrease of alpha power. While the CT-PS group initially showed the lowest global EEG power, the group differences did not reach statistical significance due to the large interindividual variability.

During the treatment period global power increased in the CT-P group ($p = 0.04$) and CT-PS group ($p = 0.005$). While in the CT-P group this increase was caused by an increase of absolute theta power ($p = 0.03$), in the CT-PS group there was also a marked increase in the fast frequency bands, absolute alpha ($p = 0.01$) and absolute beta power ($p = 0.005$). A significant reduction of relative delta power was also recorded in the CT-PS group ($p = 0.005$) (table 3). No significant changes of power were obvious in the SS and CT groups. These therapeutic effects could further be elucidated by calculating ratios between the measures obtained before and after the treatment period. As shown in figure 2, the CT-PS group significantly differed from SS and CT with respect to improved global EEG power and from all other groups with respect to decreased relative delta power (fig. 3). The ratio of relative alpha power revealed significant differences to CT-P and SS and the ratio of relative theta power was different between CT-PS and CT-P, as well as between CT-P and SS. Relative beta power did not change in the various groups.

CT-PS				SS			
before	8 weeks	16 weeks	after	before	8 weeks	16 weeks	after
20.88±4.73	23.05±4.91	22.38±5.05	22.16±6.42	20.23±4.10	21.26±5.18	20.42±5.82	19.29±5.89
4.10±1.53	3.86±1.75	4.41±1.64	4.56±1.12	4.31±1.42	4.08±1.51	4.41±1.28	4.40±1.66
6.58±2.29	7.52±1.28	6.94±1.39	6.75±20.1	6.88±2.11	7.20±0.94	7.00±1.91	7.05±1.39
3.64±7.27	3.52±6.73	1.52±2.03	1.43±2.36	2.70±4.26	2.33±2.31	2.84±4.33	3.29±4.35
3.60±1.59	–	–	4.41±1.36	4.08±1.63	–	–	4.05±1.41
5.83±4.03	–	–	5.23±4.10	5.80±5.32	–	–	4.00±5.37
33.33±6.53	–	–	37.23±5.37	32.47±5.87	–	–	36.26±5.16
27.50±7.07	–	–	32.00±7.42	27.33±9.48	–	–	32.26±8.42
12.77±5.68	11.82±6.62	13.44±6.89	12.64±5.03	10.88±4.01	9.93±5.89	11.50±5.18	9.81±5.21
2.75±2.76	–	–	2.18±2.58	3.47±2.98	–	–	3.37±3.03
5.55±1.85	6.88±1.18	7.27±1.36	6.22±1.86	5.70±2.08	6.00±1.73	5.85±1.79	5.68±2.54
7.61±2.45	8.05±2.26	7.66±2.97	7.55±2.38	6.76±2.53	6.93±2.46	6.78±2.72	7.52±2.78
0.72±0.12	0.75±0.13	0.74±0.13	0.67±0.13	0.69±0.12	0.72±0.13	0.70±0.14	0.69±0.16
60.26±33.11	–	–	79.95±43.07	107.80±126.18	–	–	68.50±42.46
319.06±57.58	–	–	328.22±60.55	322.26±63.16	–	–	317.53±65.82

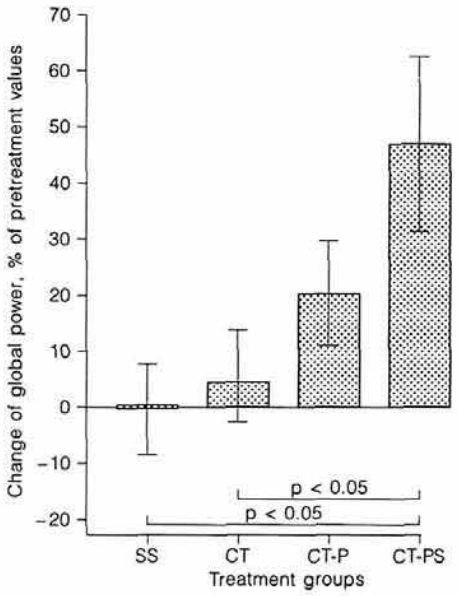
Table 3. Global power, absolute power (μVs) and relative power (%) of frequency bands before and after treatment

	Social support		Cognitive training		Cognitive training + pyritinol		Cognitive training + phosphatidylserine	
	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
Global	1,369±880	1,342±948	1,565±1,586	1,426±1,135	1,454±1,413	1,623±1,301*	874±421	1,228±579**
Delta	173.66±152.69	182.60±137.11	137.20±98.89	151.64±86.00	148.22±101.17	179.84±110.35	122.30±62.36	127.55±67.13
Theta	396.38±284.45	349.97±340.45	371.50±492.49	351.64±295.20	582.90±929.88	701.67±804.85*	247.26±122.72	322.02±137.14**
Alpha	655.35±635.69	635.04±707.95	877.09±1,069.35	774.04±900.31	574.99±502.46	590.52±554.50	365.71±276.12	590.26±446.73*
Beta	144.30±90.79	129.65±79.05	179.65±192.09	148.71±131.35	147.91±94.24	151.47±85.52	138.97±99.00	188.32±146.15**
Global and relative power								
Global	1,369±880	1,342±948	1,565±1,586	1,426±1,135	1,454±1,413	1,623±1,301*	874±421	1,228±579**
Delta	14.74±7.87	16.59±9.03	12.62±6.45	13.51±6.94	13.34±5.92	14.05±6.23	14.49±5.69	11.98±7.39**
Theta	30.36±9.70	31.26±11.34	25.74±12.33	27.97±12.80	29.87±16.54	34.75±17.94*	30.42±13.83	29.19±14.48
Alpha	42.92±15.61	40.83±18.09	47.88±17.78	47.04±18.21	42.60±15.25	38.44±15.16	39.82±15.34	43.99±19.20
Beta	11.98±5.12	11.32±5.24	13.75±7.92	11.47±6.46	14.20±10.22	12.77±7.97	15.27±6.44	14.85±6.96

* $p < 0.05$; ** $p < 0.01$.

Glucose Metabolism at Rest and during Functional Activation

The analysis was focussed on regions that are typically most affected in AD, such as temporoparietal and frontal association cortex. For comparison, also regions that are typically least affected such as primary sensorimotor cortex, primary visual cortex, putamen and cerebellum were regarded. Glucose metabolism in association cortices was reduced similarly in all treatment groups compared to age-matched controls. As reported previously [31], a sig-



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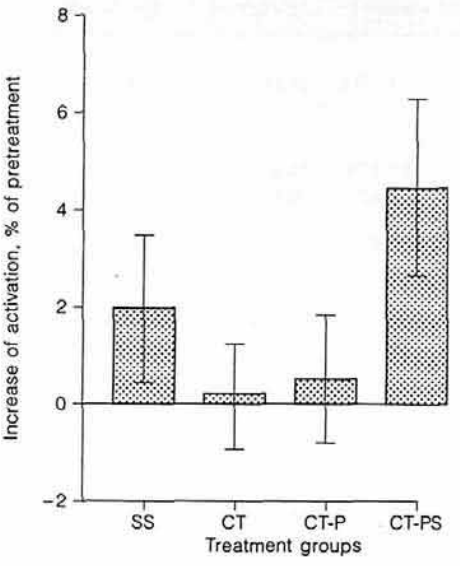

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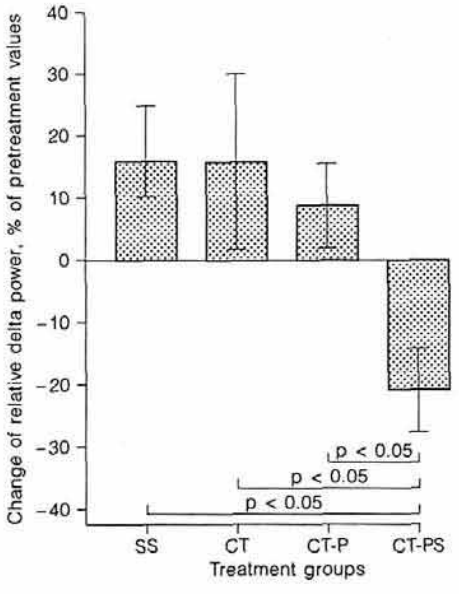

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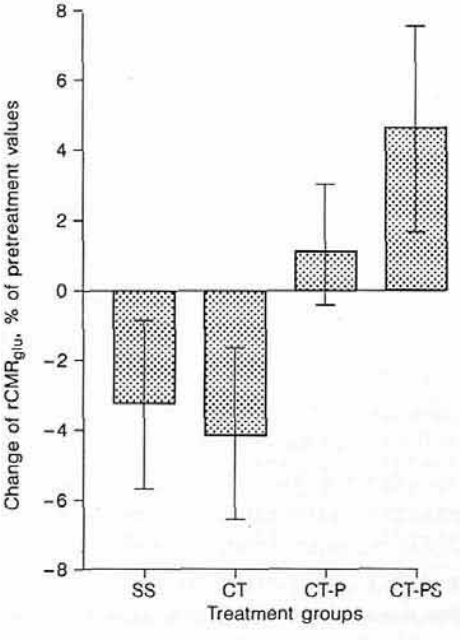

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Fig. 2. Change of global EEG power after therapy (mean ± SE) compared among treatment groups (brackets indicate significance in Tukey's multiple means comparison).

Fig. 3. Change of relative delta power after therapy (mean ± SE) compared among treatment groups (brackets indicate significance in Tukey's multiple means comparison).

Fig. 4. Change of metabolic activation by continuous visual recognition in occipital association cortex (mean ± SE).

Fig. 5. Change of resting metabolism in left superior temporal gyrus in patients with initial values below 90% of normal (mean ± SE).

Table 4. Metabolic rates before and after therapy

	Cognitive training (n = 18)	Cognitive training + pyritinol (n = 17)	Cognitive training + phosphatidylserine (n = 18)	Social support (n = 17)
Global[1]	29.87 ± 5.18[3]	29.96 ± 4.57	29.43 ± 3.92	29.26 ± 3.06
Global[2]	28.77 ± 5.03	30.55 ± 5.32	28.85 ± 4.24	29.71 ± 3.00
Global[1]	33.78 ± 5.76	32.75 ± 5.28	32.59 ± 4.91	34.25 ± 5.94
Global[2]	31.40 ± 5.40	34.21 ± 5.99	32.54 ± 5.68	32.63 ± 5.80
Frontal[1]	30.73 ± 5.39	31.38 ± 4.89	31.22 ± 6.30	30.62 ± 3.07
Frontal[2]	29.79 ± 7.05	32.43 ± 6.55	30.15 ± 6.79	31.73 ± 4.67
Frontal[1]	33.57 ± 6.89	34.58 ± 6.19	33.16 ± 7.01	35.17 ± 6.82
Frontal[2]	31.69 ± 6.23	35.60 ± 7.29	33.30 ± 7.22	34.47 ± 7.99
Temporoparietal[1]	28.39 ± 5.86	28.53 ± 4.91	29.11 ± 4.65	28.03 ± 4.91
Temporoparietal[2]	27.08 ± 6.09	28.83 ± 5.75	28.50 ± 5.42	27.90 ± 3.82
Temporoparietal[1]	31.72 ± 6.50	31.22 ± 6.29	32.18 ± 6.77	32.45 ± 6.88
Temporoparietal[2]	29.09 ± 6.02	32.07 ± 6.72	31.94 ± 7.66	30.55 ± 6.19
Primary senso.[1]	31.67 ± 3.00	32.21 ± 5.59	32.86 ± 3.80	32.54 ± 2.82
Primary senso.[2]	31.54 ± 5.04	32.98 ± 5.61	32.45 ± 4.82	33.42 ± 4.73
Primary senso.[1]	35.90 ± 5.39	36.11 ± 6.40	36.03 ± 5.15	36.99 ± 6.81
Primary senso.[2]	34.38 ± 6.17	37.29 ± 6.48	36.32 ± 5.71	36.56 ± 7.23
Cerebellum[1]	31.73 ± 4.35	30.59 ± 5.04	28.42 ± 5.41	29.85 ± 1.32
Cerebellum[2]	30.87 ± 3.63	31.40 ± 5.79	29.02 ± 5.55	29.09 ± 2.47
Cerebellum[1]	37.09 ± 7.32	33.88 ± 5.36	32.99 ± 7.01	33.78 ± 4.72
Cerebellum[2]	33.73 ± 3.82	36.64 ± 4.89	32.80 ± 6.15	32.13 ± 4.90
Putamen[1]	35.21 ± 6.25	35.72 ± 6.19	34.16 ± 5.33	34.51 ± 4.30
Putamen[2]	34.25 ± 5.37	35.97 ± 5.16	33.87 ± 6.26	35.58 ± 3.77
Putamen[1]	38.75 ± 7.01	37.59 ± 5.94	36.94 ± 6.47	39.64 ± 7.78
Putamen[2]	36.28 ± 5.41	39.93 ± 7.21	37.03 ± 6.55	37.91 ± 7.45
Visual[1]	34.60 ± 6.04	34.61 ± 6.97	35.14 ± 4.97	35.01 ± 4.79
Visual[2]	33.56 ± 6.08	35.24 ± 7.19	34.24 ± 4.63	35.94 ± 5.10
Visual[1]	42.57 ± 7.83	40.52 ± 7.61	42.87 ± 8.87	45.24 ± 10.17
Visual[2]	39.60 ± 7.78	43.14 ± 8.77	42.22 ± 9.90	42.67 ± 10.50

[1] Resting + stimulation PET before therapy.
[2] Resting + stimulation PET after therapy.
[3] Expressed in μmol/100 g/min of the absolute metabolic rate including standard deviation.

nificant correlation (r = 0.61) between severity of dementia as assessed by MMSE and $rCMR_{glu}$ in the temporoparietal association cortex was found in the whole group, and this significant relationship persisted throughout the study at rest as well as during activation.

Metabolic rates at rest and during activation were comparable before and after treatment among the four groups (table 4). There were no significant differences between groups with regard to changes of metabolism (values after treatment divided through initial values) in the target regions described above. To detect metabolic correlates of the neuropsychological and electrophysiological differences between groups, additional regions of interest including the occipital association cortex were analyzed. After treatment with CT-PS, the increase of metabolism in the visual association area during functional activation, normalized to the increase in the primary visual cortex, was significantly larger than before treatment (p = 0.026, fig. 4), whereas there was no such increase in the other groups. Additionally, an effect of this treatment strategy on resting relative $rCMR_{glu}$ could be demonstrated in temporal regions with low initial values by an analysis of covariance with the pretreatment values as a covariate (p = 0.01 for the interaction between group and covariate). The effect is illustrated in figure 5, showing an increase of metabolism in CT-PS patients with initial values below 90% of normal in the left superior temporal gyrus.

Discussion

The results of this study in which four groups of AD patients under various forms of therapeutic management were followed for 6 months are disappointing, but nevertheless they are in accordance with previous experience: the course of cognitive decline cannot be influenced substantially by symptomatic therapeutic strategies. The effects of such treatments are short-lasting and they necessitate meticulous examination and careful statistical analysis of large patient populations. The study indicates, however, that certain drugs such as phosphatidylserine may help to ameliorate cognitive and memory impairment in these patients, and these albeit transient effects are associated with improved electrophysiological power of the brain, especially decrease of relative delta power, and increased glucose utilization in some relevant brain regions. In this context it is noteworthy that the beneficial effect of such symptomatic treatment could be shown concordantly in three independent measures of brain function: neuropsychological testing, quantitative EEG recording, and assessment of glucose metabolism. The coherence of the various functional assessments was also emphasized by the significant correlation of changes in MMSE and in activated glucose metabolism in the whole group.

Large-scale drug studies in AD are usually restricted to rather short periods of this slowly progressive disorder limiting the treatment and observation periods to 6–12 weeks, e.g., tacrine for 6 weeks [5], tacrine and lecithin for 6 weeks [50], tacrine for 12 weeks [6], physostigmine for 6 weeks [51], oxiracetam for 4–12 weeks [52]. Trials designed for longer observation periods are mostly restricted to smaller patients populations (levocarnitine 3 plus 3 months [7], piracetam 1 year [11]) and the effects of treatment were evident only in subtests (levocarnitine [7]), indicated as a trend to slow the progression of the disease (piracetam [11], indomethacin [53]) or demonstrable only in a rather small number of responders (bifemelane [54], tacrine [50]). The influence of memory training or cognitive rehabilitation programs were usually followed for longer periods, but gains have been generally small or nonexistent [13] and the effects seen in only a few studies were restricted to persistence of performance in everyday cognitive and memory tasks and daily life activities compared to a decline in these tasks in the untreated control groups [55–57].

Since previous investigations of our group have indicated an effect of several drugs on brain glucose metabolism and cognitive performance when given for 2–6 weeks [58–60], a parallel 4-group trial was designed to evaluate the efficacy of several therapeutic strategies over 6 months, which is to be considered a meaningful period for a chronic progressive disorder like AD.

The effect of PS was best visible in cognitive tests after 8 weeks corresponding to the short-term efficacy of other therapeutic interventions, where an effect only in responders was also evident [5, 6]. The therapeutic effects faded later during the progression of the disease, making it more difficult to prove the action on quantitative measures such as glucose metabolism. However, it was shown previously [60, 61] that PS can increase $rCMR_{glu}$ diffusely when given for several weeks, and this increase is finally cancelled out by the progressive deterioration in the course of the disease leading to further cognitive and concomitant metabolic impairment [62]. A significant improvement of metabolic response to specific tasks, however, was still demonstrable after 6 months, and a specific increase of markedly reduced resting metabolism in temporal regions was evident after PS treatment. These effects concerned brain areas involved in the processing of visual information – the occipital association area most demanded in the applied activation task – and in intermodal interaction (temporal gyri for storage of visual information and verbal interpretation). It cannot be deducted from our study, whether the effect of PS, which was clearly demonstrated in chronic animal experiments [63, 64] and in clinical studies [21, 22], is due to its action on the cholinergic system – a mechanism responsible for the therapeutic efficacy of most drugs used for symptomatic treatment in AD – or to another of its multiple activities. In our data, there is no indication that cognitive and/or memory training has a beneficial effect on symptoms in AD. In accordance with previously reported results [13], cognitive training was not better than social support in our study, and the addition of a drug activating brain and cholinergic activity, as pyritinol [23, 24] had less effect on neuropsychologic performance and EEG power, and no significant influence on glucose metabolism after 6 months. While CT and pyritinol was better than CT alone, with a few exceptions (e.g., EEG analysis) these differences did not reach statistical significance in our small samples.

It can be deducted from the results presented in this study that quantitative EEG analysis is a useful tool for showing the effects of treatment in the course of AD. Such noninvasive examinations can be repeated in short intervals at low cost and with comparably uncomplicated equipment, especially as correlations among various quantitative EEG abnormalities and severity of cognitive decline have been described previously [49, 65–68]. It is unclear which of the quantitative EEG measures, de-

crease of fast or increase of slow frequency band power, is more useful as marker of dementia progression. The results of EEG recordings, however, do not yield highly regional data and are unreliable with respect to differential diagnosis of cognitive impairment [69]. For improved differential diagnosis, especially in patients with mild cognitive decline and for assessment of severity of dementia quantitative measurement of regional metabolism or flow is the procedure of choice [70, 71] by which regional effects and improvements in the reserve capacity of the brain to cope with increased demands during functional activation can also be evaluated.

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