ITALFARMACO S.p.A.

FINAL REPORT

CHOLINE ALPHOSCERATE BY PARENTERAL AND ORAL ROUTE IN THE TREATMENT OF DEMENTIA OF THE ALZHEIMER TYPE. (STUDY COL/95/1)

GCP Statement: the study was conducted according to Good Clinical Practice (GCP) procedures.

Date of Report: July, 24th, 2001

CLINICAL STUDY REPORT

Date of Report:	July 24th, 2000
Status:	Final
Study Title:	Choline Alphoscerate by Parenteral and Oral Route in the Treatment of Dementia of the Alzheimer Type.
Investigational product:	Choline Alphoscerate
Protocol No:	COL/95/1
Phase:	III
First Patient in:	December 1998
Last Patient out:	February 2000

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STUDY SYNOPSIS

Name of Sponsor/Company: Italfarmaco S.p.A. Milan, Italy

Individual Study Table Referring to part of the Dossier: *(For National Authority Use only):*

Name of Finished Product: Gliatilin

Volume:

Page:

Name of Active Ingredient: Choline Alphoscerate

Title of Study: Choline alphoscerate by parenteral and oral route in the treatment of Dementia of Alzheimer type.

Investigators: 1) Maria De Jesus Moreno M.D., 2) Ernesto Garcia Herrera M.D., 3) Noe Saul Barroso Rodriguez 4) Fernando Ville Corona M.D.

Study Centres: 1) Instituto Nacional De La Senectud, Mexico City. 2) Hospital San Juan De Aragon. Unidad De Geriatria, Mexico City. 3) Hospital De Especialidades. Centro Medico "La Raza", Mexico City 4) Centro Medico Union, Guadalajara.

Publication (reference):

Studied period (years): 1998 - 2000

Phase of development: Phase III (Multicenter Clinical Trial)

Date of first enrolment: December 1998

Date of last completed: February 2000

Objectives: To demonstrate that choline alphoscerate given by parenteral route (intramuscolar) and then by oral route is effective and safe in counteracting attention and memory impairment in patients with Dementia of the Alzheimer type (SDAT).

STUDY SYNOPSIS

Methodology: Intramuscolar administration of vials containing 1000 mg of choline alphoscerate for 90 days, followed by oral administration of capsules containing 400 mg of choline alphoscerate for 90 days to patients with SDAT that fulfill inclusion and exclusion criteria.

Number of patients (planned and analyzed): Planned 220 Enrolled: 57 Analyzed: 57

Diagnosis and main criteria for inclusion: Diagnosis of SDAT according to DSM IV and NINCDS-ADRDA criteria, Hachinski Score (HIS) < 4, Mini mental State Examination(MMSE) between 12 and 26, Global Deterioration Scale (GDS) between 3 and 5, Hamilton Depression Scale (HAM-D) less than 22, witten informed consent.

Test product, dose and mode of administration, batch number:

Choline alphoscerate: 1 injection daily, intramuscolar
Lot. No. Prova C/1
Choline alphoscerate: 3 capsules daily, oral
Lot. No.U0010800

Duration of treatment: 90 days i.m., followed by 90 days oral

Criteria for evaluation:

Efficacy: The primary end-point is the modification of the ADAS Cog. Score observed at the end of study period. Secondary end-points are the changes in the ADAS Behav. and Total, MMSE, GDS, Clinical Global Impression (CGI) and Global Improvement scores.

Safety: Adverse events observed during the study period are the primary endpoint, while the Physician's Evaluation of tolerability (poor, acceptable, good, very good) during the 180 days of treatment with the study drugs is the secondary end-point.

Statistical methods: Descriptive statistics, Maximum likelihood estimates of parameters in a mixed –linear model, Ranks transformation, Cochran-Mantel-Haenszel test.

STUDY SYNOPSIS – CONCLUSIONS

EFFICACY RESULTS

Adas Cog.

In the treated patients the raw mean ADAS Cog. Score decreased after 90 days (end of the intramuscolar treatment period) with respect to baseline, and this decrease continued during the following 90 days of the oral treatment period of the study.

Mean decrease in ADAS Cog. Score after 90 days of i.m. treatment was 6.82 points, while at the end of the study, after 3 months of oral treatment, it was 9.83 points in comparison with baseline.

Estimated ADAS Cog. Score contrast was obtained as results from the Mixed Linear Model Analysis within treatment using ITT populations.

A statistically significant decrease in ADAS Cog. Score ($p<0.0001$) with respect to baseline scoring was observed after 90 days and 180 days.

No statistically significant differences in ADAS Cog score were observed between the end of the i.m. treatment and the end of the of oral treatment.

Psychometric evaluations

MMSE score increased by 4.31 points after 90 days and by 6.01 points after 180 days with respect to baseline.

GDS stage decreased by 0.7 points after 90 days and by 1.07 points after 180 days with respect to baseline.

ADAS Behav. score decreased by 3.63 points after 90 days and by 4.15 points after 180 days with respect to baseline.

ADAS Total score decreased by 10.46 points after 90 days and by 14 points after 180 days with respect to baseline.

CGI score decreased by 0.43 points after 90 days and by 0.96 points after 180 days with respect to baseline.

Estimated MMSE, GDS, ADAS Behav. and Total and CGI Score contrast were obtained as results from the Mixed Linear Model Analysis within treatment using ITT population.

Statistically significant changes in MMSE, GDS, ADAS Behav. and Total and CGI scores ($p< 0.0001$) with respect to baseline scoring were observed after 90 and 180 days.

Global Improvement

Global Improvement score was 2.21 ±1.01 at day 90 and 1.9 ±1.04 at day 180 with an decrease by 0.31 points after 180 days with respect to day 90.

Patients Termination Records

99.2% of patients completed the study and took the drug as prescribed or without relevant irregularities.

No significant clinical events were observed in a substantial proportion of patients during the study period.

When patients were asked if they wished to continue the i.m. treatment with the study drug, 54 patients out of 57 gave positive answers: 13 patients were inclined to say yes and 41 patients said definitively yes. When patients were asked if they wished to continue the oral treatment with the study drug, 50 patients out of 57 gave positive answers: 9 patients were inclined to say yes and 41 said definitively yes.

SAFETY RESULTS

Adverse events

Overall, fifty-one adverse events were reported, in 21 patients (26 in 16 patients with i.m. vials and 25 in 17 patients with oral capsules); 29 were considered drug related, in 18 patients (15 in 11 patients with i.m. vials and 14 in 9 patients with oral capsules).

Out of these 51 adverse events, no one was classified as serious.

No patient was withdrawn early from the study due to adverse drug reactions.

With both formulations of choline alphoscerate, adverse events/reactions were mainly related to the CNS and gastrointestinal system; their severity was mild in most of patients.

Physician's Evaluation of Tolerability

The most frequent rating was " Good" both for vials (47.3 %) and capsules (50.9 %).

CONCLUSIONS

The positive efficacy results gained in this open, uncontrolled study are to be cautiously evaluated, as they were not be properly compared with a no-treatment situation.
Nevertheless, two different points can be suggested, to give some relevance to the efficacy results of this study:

- The study results could be evaluated in comparison with published longitudinal studies in non treated SDAT patients, in which the natural worsening of SDAT over the time is described;
- anyway, the efficacy results of this study could be regarded as "confirmatory" of the clinical results gained in previous published trials with choline alphoscerate, and in particular observed in Study COL/95/2 (double blind, placebo controlled study in mild to moderate SDAT patients recently completed by the same Investigators of this trial).

According to the data on treatment side effects collected in this study, both the administered formulations had a good profile of tolerability, and can be considered safe and suitable for a long-term treatment. In particular it must be stressed that, as for incidence, type and severity of adverse reactions, the clinical tolerability of the i.m. formulation of choline alphoscerate resulted comparable to that of the oral form.

In conclusion, the results of this open, multicenter, non controlled trial confirm that choline alphoscerate i.m vials and oral capsules are safe and effective in the long term treatment of mild to moderate Dementia of the Alzheimer Type.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADAS:	Alzheimer Disease Assessment Scale
ADAS Cog.:	Alzheimer Disease Assessment Scale - Cognitive
ADAS Behav.:	Alzheimer Disease Assessment Scale – Behavioral
A.M.I.C.:	Asociación Mexicana para la Investigación Clínica. (Mexican Association for Clinical Research).
CAT:	Computerised Axial Tomography
CGI:	Clinical Global Impression
C.I.:	Clinical Improvement
DSM IV:	Diagnostic and Statistical Manual
ENT:	Eye, nose, throat
GCP:	Good Clinical Practice
GDS:	Global Deterioration Scale
HAM – D:	Hamilton Depression Rating Scale
I.M.	Intramuscular
IRB:	Institutional Review Board.
M.HIS:	Modified Hachinski Ischemic Score
MMSE:	Mini Mental State Examination
NINCDS-ADRDA:	The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
SDAT / AD:	Senile Dementia of the Alzheimer's Type
VAD	Vascular dementia

1. INTRODUCTION

Memory decline and mild impairment of cognitive functions may occur in healthy individuals during the later decades of adult life. Memory loss and impairment of cognitive performance are likely to be associated with specific disorders, such as SDAT, other neurodegenerative disorders and vascular dementias.

Alzheimer's disease (SDAT) is the most common of the primary dementias in the elderly. It is a degenerative brain disease, characterized by progressively impaired cognitive capacity. Memory deficits are the most prominent symptom, often accompanied by deterioration of language skills, disorientation, impairment of judgement, and emotional and behavioral disturbances.

SDAT afflicts as many as 4 million people in the USA, 1.3 of whom are categorized as having severe SDAT (1). Men and women appear to be afflicted in equal numbers. The disorder is strongly correlated with increasing age: while SDAT affects 10.3 % of persons over 65 years old, it is present in 47% of those aged 85 years and over (2). SDAT impacts negatively upon life expectancy (3), the disease now being the fourth leading cause of death among adults, after heart disease, cancer and stroke.

In 1907 the first description of SDAT was published (4); over the years, the devastating effect of SDAT has been more fully acknowledged and the social impact of the disease could increase even further, with the increase in the elderly population that at present is growing 2.5 time faster than the population at large.

SDAT is already perceived as a major public health problem. In addition to severe disabilities in patients and the emotional burden upon family members, the social costs of SDAT are substantial. Although estimates vary, SDAT has an estimated annual cost of over $ 90 billion in USA (5). The impact is expected to increase significantly in the future. As the population ages, the number of people with this disorder is likely to grow to 14 million the middle of the 21st century, unless effective measures for its treatment or prevention are discovered.

Among the neurotransmitter systems, the impairment of the cholinergic pathway plays an important role in the development of signs and symptoms of cognitive dysfunction. The observations of the loss of cholinergic function in neocortex and hippocampus of SDAT patients was the rationale to develop the hypothesis of cholinergic

replacement of therapy patients (6). Among the possible approaches to enhance impaired cerebrocortical cholinergic neurotransmission, inhibition of endogenous ACh degradation through ChE inhibitors and precursor loading were the most largely investigated in clinical trials (7-9). Despite encouraging early results, (10,11) well-controlled clinical trials not always showed significant clinical improvements in patients treated with choline or lecithin (12-17).

AChE inhibitors have been widely studied in the last years as cognitive-enhancing treatment for SDAT; they provide a time-dependent improvement in symptoms of AD and possibly stabilize or slow for some time the decline of cognitive function and daily living activities (7).

Treatment with AchE inhibitors is at the present an important step in the treatment of the disease; however a retrospective analysis of available clinical trials with these drugs did not confirm always a significant benefit on all four key symptom domains of AD such as cognition, behavioural disturbances, daily living activities and global function (7).

Choline alphoscerate (L-α-glycerylphosphorylcholine, Gliatilin) is a semi-synthetic derivative of phosphatidylcholine; pre-clinical studies have demonstrated that choline alphoscerate increases the release of acetylcholine in rat hippocampus (18), facilitates learning and memory (19), improves brain transduction mechanisms (20), decreases the age-dependent structural changes occurring in the rat frontal cortex and hippocampus (21), positively influences membrane fluidity (22), improves cognitive deficit in experimental models of aging brain (23,24) and reverses mnemonic deficits induced by scopolamine administration (19).

The clinical efficacy of choline alphoscerate in controlling the symptoms of SDAT and vascular dementia (VAD) was confirmed on a large number of patients treated with the drug in controlled trials versus reference drugs or placebo.

Ten clinical studies (25-34) on the effect of choline alphoscerate in adult-onset dementia disorders have been published, involving 1,570 patients. The results obtained with choline alphoscerate were superior or equivalent to those observed in control groups under active treatment, and superior to the results observed in placebo groups. Both in SDAT and in VAD, administration of choline alphoscerate significantly improved patient clinical condition; the drug was effective especially, but not only, on cognitive symptoms (memory, attention).

The clinically significant improvement observed in different studies demonstrates that choline alphoscerate is different from other cholinergic precursors, such as choline or lecithin, that provide only minor evidence of improving cognitive impairment typical of SDAT and VAD (10-17).

Among the data gathered up to date in different situations in which the drug was tested, these results are the most relevant ones:

- the significant activity of choline alphoscerate on cognitive symptoms such as memory and attention, which characterise the clinical picture of dementia disorders;
- the very low incidence of adverse events, that is, the high tolerability profile of this drug.

This multicentre study was performed to evaluate whether i.m. treatment followed by oral treatment with choline alphoscerate was safe and effective in the treatment of SDAT. This study was conducted and monitored by the Asociación Mexicana para la Investigación Clínica, A.C. (A.M.I.C.) according to the request of the Coordinator, who acted on behalf of the Sponsor Italfarmaco S.p.A., Milan, Italy.

2. ETHICS

The study was conducted in accordance with the Declaration of Helsinki and amendments (Tokyo 1975, Venice 1983, Hong Kong 1989 and Somerset West - South Africa 1996) in accordance with local and international regulatory requirements and with the approval of the Mexican Board of Health.

The study initiated after the protocol was approved by the IRBs or a relevant authority (Region, Hospital, University). Protocol amendment No.1 was also approved by the IRBs of each participating Center.

Before starting any related study evaluation, patients or a legal representative were informed about all the study procedures; the informed consent was clearly explained to them and the patients or the legal representative signed before enrollment.

A copy of the informed consent is attached in appendix 14.1.2.

3. INVESTIGATORS AND ADMINISTRATIVE STRUCTURE OF THE STUDY

The study Coordinator was Phidea SL represented by Vincenzo Cocuzza, M.D. The Asociacion Mexicana para la Investigación Clinica (AMIC) was hired by Phidea SL in order to organize, coordinate, manage and monitor this study in Mexico.

This multicenter study was conducted in 4 different Centers located in 2 cities (Mexico City and Guadalajara). A list of Investigators and Co-investigators with their qualifications, affiliation and role in the study is attached in Appendix 14.1.6.

The clinical lab "Carpermor Laboratorio de Referencia Internacional" located in Mexico City was the reference lab for Centers no. 1, 2 and 3, while the clinical lab. "Icardi", located in Guadalajara was the reference lab for Centers no. 4. The two laboratories involved in the study had standardised methods and technical procedures according to international Quality Standards (App.14.1.8) .

Study drugs, Case Report Forms, Drug Accountability Sheets, copies of the informed consent, as well as instructions about the procedure of blood samples collection were provided to the Investigators by A.M.I.C.

A meeting with the Investigators and Co-investigators of the Centers was held to uniform criteria and manage the logistics of the study; the Investigators and Co-investigators were trained according to the protocol by the monitor of the study.

The statistical analysis was performed by Phidea S.p.A., Milan, Italy.

Note: the objective of the study and the investigational plan are presented on paragraph 4, 5.1, 5.2 and 5.3 as described in the original protocol; that is not including changes made by Protocol Amendment No. 1. These changes are described on paragraph 5.4.

4. OBJECTIVE OF THE STUDY

According to the original protocol, the study was aimed to demonstrate that choline alphoscerate given parenterally (i. m.) is more effective than placebo in counteracting attention and memory impairments in patients affected by Dementia of Alzheimer Type (SDAT).

5. INVESTIGATIONAL PLAN

5.1 Overall design of the study and plan

5.1.1 Study Design

It was planned as a placebo controlled, randomized, double blind, multicenter clinical trial evaluating two parallel treatment groups.

5.1.2 Discussion of the design

Study period

According to the original protocol, 90 days of i.m. treatment were expected to be adequate to achieve the primary end-point of the study (ADAS Cog. Score decrease), and to evaluate the clinical safety of choline alphoscerate .

Blinding

Blinding of this study was to be ensured by administering identical vials of choline alphoscerate and placebo (See 5.2.6.).

5.1.3 Study Population

5.1.3.1 Patient Population

According to the original protocol, plans were made to recruit 220 patients with probable or possible SDAT and randomly allocate them to one of the two groups of treatment (active or placebo).

5.1.3.2 Inclusion Criteria

-Age up to 80 years

-Clinical history of progressive impairment, from 60 to 80 years of age.

-Diagnosis of probable or possible SDAT according to DSM IV and NINCDS-ADRDA criteria.

-Mini Mental state examination (MMSE): 12-26

-Modified Hachinski Score less than 4

-Hamilton Depression Scale (HAM-D) below 22

- Subjects living with a relative who was able to play an active role in the performance of the study (informed consent, understanding of drug administration timing and

compliance). When the patient lived in an Elderly Residence, the eligibility was evaluated case by case.

- School education: at least five years. Patients able to read and write and to fully understand the requested psychometric evaluations and to sign, together with his/her accompanying person, the Informed Consent.
- Patients in good general clinical conditions, as assessed by clinical history, clinical/neurological examinations and laboratory tests.
- A cerebral CAT performed within the last six months, showing a normal image or compatible atrophy.

5.1.3.3 Exclusion Criteria

-Presence of the following neurological diseases: congenital and/or acquired metabolic encephalopathies, toxic and drug induced encephalophathy, Parkinson disease, MID, Stroke, Epilepsy, CNS infective diseases, CND demyelinating disease, CNS heredodegenerative diseases.

-Neoplastic and/or traumatic lesion of the brain.

-Folates and/or B 12, B1, B6 deficiency; severe anemia (HB < 9 g/dl).

- Presence of psychiatric diseases, malignant neoplasm, AIDS, severe organ failure (heart, liver, kidney, lung), severe diabetes, endocrine disorders.
- A score of 22 or higher on the 22-item Hamilton Psychiatric Rating Scale for Depression

-Alcoholism or drug addiction

- Treatment in the previous 30 days with neuroleptics (excluding thioridazine), antidepressants, methyldopa or nootropics drugs. Treatment in the previous 90 days with reserpine and/or clonidine.
- Low compliance with drug treatment.

5.1.3.4 Removal of Patients from Therapy or Assessment

Patients were to be withdrawn from the study if any of the following conditions occurred: the recruitment diagnosis resulted to be incorrect (incorrect diagnosis), at least one of the conditions listed as exclusion criteria were observed (protocol violation) during the study period, emerging symptoms of such a nature or severity to

suggest the withdrawal of the patient (adverse events), death, patient not available for the final visit for any reason (complications, other illnesses, surgery, spontaneous withdrawal, withdrawal of consent, etc.).

Patients withdrawn from the study, regardless of the reason, were not to be replaced.

5.2 Study Treatments

5.2.1. Preparation, Packaging, Labeling

According to the original protocol, the test medications were choline alphoscerate vials 1000 mg per unit dose and identical placebo vials. The drugs were packaged in properly labeled boxes. The label showed the patient's number, the assigned treatment number according to the randomization list and the legend "For clinical research only", together with the expiry date, batch number, code of the study, and daily dosage.

5.2.2. Drug Storage, Dispensing, Inventory

The study drugs were stored in a dry place, at room temperature and accessible only by the person in charge of dispensing to the patients.

Investigators prepared an inventory of the test medications where the amount and date of the study material received, as well as the number of vials administered to each patient, were noted.

5.2.3 Drug Administration. Selection of Dose per Patient

The dosage of the drugs was to be 1 i.m. vial daily of choline alphoscerate (corresponding to 1,000 mg/day) or identical placebo vials for 90 days.

The administered daily number of vials was to be the same for all the treated patients.

5.2.4. Identification of the Investigational Product

Choline alphoscerate vials containing 1000 mg per unit dose, batch number Prova C1 and placebo vials, batch number Prova C1.

5.2.5. Patients Allocation to Treatment Groups

The patient's allocation method and a balanced computer-generated randomisation list were provided by Phidea S.p.A (Appendix 14.1.7). Phidea S.p.A kept a master code list.

5.2.6. Blinding Procedure

The Sponsor prepared vials containing choline alphoscerate and placebo, identical in size, shape and color.

5.2.7. Previous and Concomitant Treatments

The Investigator paid attention to exclude concurrent pharmacological treatments that might have interfered with the appropriate evaluation of the effects of the study drug. In particular, neuroleptics (excluding thioridazine), and other drugs which have an intrinsic psychotropic activity were avoided.

The use of insulin, ACE inhibitors, calcium entry blockers (other than nimodipine), and coronary vasodilators was allowed, provided the treatment had been stable for more than 3 months before initiation of the study.

Other concomitant medications necessary for the treatment of medical diseases other than Dementia of the Alzheimer Type were permitted at the discretion of the Investigators. Patients were instructed to inform the Investigator before taking any medication during the period of the study. Permitted concomitant medications administered at baseline or at any planned visit were recorded in the appropriate section of the Clinical Record Form (CRF).

5.3 Study Procedures - Visit Schedule and Assessments

According to the original protocol, the study had a screening period of two weeks and a treatment period of 90 days.

Before the start of treatment (T0), a screening examination was to be conducted on each patient who appeared to be an eligible case. To confirm the inclusion criteria in each patient, the general and neurological history, a physical and neurological examination and a CAT (if not already performed during the 6 months preceeding the trial entry) were to be performed.

M-HIS, MMSE, GDS, HAM–D, ADAS Score (Cog., Behav. and Total), CGI, Lab parameters and vital signs were evaluated at screening or T0 in each patient.

The eligible patients were to be treated i.m. for 90 days during which randomised choline alphoscerate or placebo vials were to be administered blindly. After the T0 visit, the next one was at day 90 (Final visit) during which MMSE, GDS, ADAS Score (Cog., Behav. and Total), CGI and C.I. were to be evaluated.

5.3.1 Efficacy Assessments

The primary end-point of efficacy was the modification of the score to be obtained in the cognitive section of the Alzheimer Disease Assessment Scale (ADAS Cog.) at T90.

Secondary end-points of efficacy were the modifications of ADAS behaviour and total score, the stage of GDS, the MMSE score, the score of the Clinical Global Impression and the score of the Global Improvement at T90.

A patient termination record was to be collected at the end of the study period.

During the time-course of the study the evaluations of efficacy were to be performed by the same Investigator and by preference in the same conditions.

5.3.2 Safety Assessments

The primary end-point of safety consisted in the adverse events observed during the study periods. The type, severity, time of onset, duration, frequency, relation to study drug of emerging adverse events were reported in the Clinical Record Form. Moreover, other relevant clinical observations, concomitant diseases, action(s) required, outcome and the supposed cause(s) were reported. If the event was judged as serious (fatal, life-threatening, which involved or prolonged hospitalization or resulted in persistent or significant disability) the Investigator had to inform the study Monitor within 24 hours by faxing the "Serious Adverse Event" Form.

Laboratory abnormalities were not reported as adverse events unless they were accompanied by a clinical diagnosis or produced clinical signs or symptoms different from dementia symptoms.

Another parameter of safety was the final Investigator's evaluation of tolerability assessed according to a 4 point scale (poor, acceptable, good, very good).

5.4.1 Protocol Amendment No. 1

Two different studies were planned to confirm the safety and efficacy of choline alphoscerate in patients with Dementia of the Alzheimer Type.

Present study COL/95/1 was planned to evaluate, in a double blind comparison vs placebo, the efficacy and tolerability of choline alphoscerate vials given i.m. for 90 days in patients affected by Alzheimer's disease. It was planned that at the end of this study, the patients giving a further informed consent could be admitted to study COL/95/2, which evaluated (in a double blind comparison vs. placebo) the efficacy and tolerability of choline alphoscerate given orally for three months.

The organization of the studies was initiated in January 1998, by identifying possible trial sites and Investigators. After study approvals had been obtained in April and May 98, the study drug administration was scheduled to start in September 98. At the end of November 98, 65 patients had been screened in 9 active trial sites, but only 4 patients were on treatment with study drug in protocol Col/95/1.

The main obstacle to obtain the patient's consent to participate in the COL/95/1 study was the three month i.m. treatment with placebo; i.e., most of the selected patients refused to participate, as they were made aware of the possibility of receiving intramuscular administration of 4 ml of placebo daily.

To overcome this problem, a protocol amendment for both Col/95/1 and COL/95/2 studies (Amendment No.1 – App.14.1.3) was submitted and approved by each IRB between January and May 1999.

According to this amendment, in present study COL/95/1 patients were not randomised to treatment with placebo, but all of them received unblinded i.m. choline alphoscerate- 1 vial/day for 3 months, followed by 3 months unblinded oral treatment with the same drug (3 capsules/day).

Psychometric evaluations (MMSE, ADAS, GDS, CGI and Global Improvement) were performed at baseline, after 90 days (end of the intramuscolar phase) and after 180 days of treatment (end of oral phase and of the trial). Patient compliance was to be checked every month during therapy.

Patients who refused their consent to receive i.m. active treatment were immediately asked to switch to study COL/95/2.

Following this Amendment, Investigators were provided with oral capsules containing 400 mg of choline alphoscerate, and placebo vials were re-collected from Investigational Centers

The capsules of choline alphoscerate were packaged in properly labeled boxes. The label showed the patient's number and the legend "For clinical research only", together with the expiry date, batch number, code of the study, and daily dosage. Capsules were stored in a dry place, at room temperature and accessible only by the person in charge of dispensing to the patients. Investigators prepared an inventory of the test medications, on which the amount and date of the study material received, as well as the number of capsules administered to each patient, were recorded.

The dosage was of 3 capsules daily, corresponding to 1,200 mg/day of choline alphoscerate for 90 days. Their batch number was U0010800.

TABLE 1 – VISIT SCHEDULE AND ASSESSMENTS

ASSESSMENT (DAY)	**-15/Bas**	**30**	**60**	**90**	**120**	**150**	**180**
Informed Consent	X						
History	X						
Physical/Neurological Exam	X						
CAT*	X						
Mod.Hachinski Ischemic Scale	X						
Hamilton Depression Rating Scale	X						
ADAS	X			X			X
MMSE	X			X			X
GDS	X			X			X
CGI	X			X			X
Clinical Improvement				X			X
Adverse Events		X	X	X	X	X	X
Lab parameters	X						
SBP, DBP, HR	X						
Compliance	X	X	X	X	X	X	X
Patient Termination Record							X
Physician's evaluation of tolerability							X

* If not already performed during the 6 months preceeding the trial entry

Treatment:
Day 0 – 90 i.m. choline alphoscerate (1 vial/day)
Day 91 – 180 oral choline alphoscerate (3 capsules/day)

6. Data Management

6.1 Data Collection and Audit to Study Centers

Periodic monitoring visits by authorized representatives of A.M.I.C. were planned with the Investigators to review the study documentation. During these visits the Investigator and the monitor reviewed the progress of the study and compliance with the study protocol, discussed any problems, reviewed the CRFs for legibility, accuracy and completeness of data, checked the patients' diary and the results of lab analyses.

The study data were recorded in the Clinical Record Forms according to the following instructions: a black ink ball point pen was used to write the data on the CRFs, which were filled-in only by the Investigator or a duly authorized person. If a mistake in data entry was made, the wrong datum was crossed out and the right one was entered together with the signature and the date. At the completion, the Investigator signed each CRF, ensuring the completeness of the data.

Study Center no. 1 was audited on 28th February 2001 and study Center no. 2 was audited on 1st March 2001 (App. 14.1.9).

6.2 Data Base Management and Quality Control

Data items from the Clinical Record Forms were entered into the study database.

Subsequently, the information entered into the database was systematically checked by data Management staff. Obvious errors were corrected by staff personnel while other errors or omissions were returned to Investigational Sites for resolution. The resolution was entered in the database.

Quality control audits of all key safety and efficacy data in the database were performed to ensure that the database met the set quality standards. When the database was declared to be complete and accurate, it was locked.

7. Statistical Methods and Assessment of Sample Size

7.1 Software

The statistical analyses were carried out by Phidea S.p.A. using SAS software program release 6.12 for PC windows. SAS Statistical Procedure were validated in agreement with SAS STAT Handbook.

7.2. Sample Size Determination

In the original protocol, the sample size was calculated on the basis of the results obtained in a trial with Tacrine (35) published a few months before COL/95/1 and COL/95/2 studies were planned, in order to detect a mean difference in ADAS Cog. Score of 2.2 points between the group treated with choline alphoscerate and the one treated with placebo at the end of treatment .

The Standard Deviation of this difference, observed in the former study on Tacrine was estimated as 6 points.

It was calculated that a sample size of 95 patients per group would be required to detect a significant difference between the two groups, assuming one tail α=0.05 and β=0.2 with a potency of 80%. Considering a drop-out rate of about 10%, it was estimated that at least 190 patients (95+95) +10%=220 would have to be enrolled.

Following amendment No. 1, no sample size calculation based on statistical hypotesis was feasible. Therefore, it was agreed with Investigators that they would have been including into the study as many elegible patients as possible, during about one year period.

7.3 Statistical Procedures

1) Descriptive statistics
2) Maximum likelihood estimates of parameters in a mixed –linear model
3) Ranks transformation
4) Cochran-Mantel-Haenszel test.

7.4 Analysed Variables

Baseline demographic variables, risk factors and relevant clinical variables were summarized descriptively to characterize the study population. In general, arithmetic mean, standard deviation and range were provided for continuous data, whilst the frequencies of categorical data were tabulated.

Statistical hypotheses concerning primary and secondary efficacy end-points were tested using mixed model estimates among visits as fixed effect, and a non parametric covariance matrix (unstructured) as nuisance parameters (36).

Furthermore, to satisfy gaussian assumptions underlying the mixed linear model, GDS, CGI and GI variables expressed by a 7-point ordinal scale were transformed into ranks according to Conover and Iman.

The physician's judgement of treatment efficacy at the end of trial was analyzed using the Cochran Mantel Haenszel test.

Tolerability and safety data were summarized by descriptive statistics. The physician's judgement of treatment tolerability at the end of trial was analyzed using the Cochran Mantel Haenszel test.

7.5 Analyzed Population

Completers population: eligible patients who completed treatment and scheduled visits. These patients were evaluated with Per Protocol analysis for the primary endpoint of efficacy.

All available data population: including all enrolled patients regardless of study completion. These patients were evaluated with Intention to Treat analysis.

7.6 Handling Missing Data

Statistical methods described above use all available data regardless of patient's study completion. Further, maximum likelihood estimates of general mixed linear models are unbiased in presence of a MAR mechanism of missingness (MAR⇒ drop-out probability depending on previous response).

8. STUDY PATIENTS

8.1 Baseline Disposition of Patients

Following the amendment of the study design, 57 patients with probable Dementia of the Alzheimer Type were enrolled in this study (Table 2).

Eleven patients were considered protocol violators (Table 3 and App.14.3.2).

One of these 11 patients was a protocol violator, was lost to follow-up and was also a low compliant.

57 patients were considered for Intention to Treat analysis, 46 patients for Per Protocol analysis.

TABLE 2 - DISPOSITION OF THE PATIENTS

	CHOLINE ALPH.

ENROLLED	57
COMPLETED	56
PROTOCOL VIOLATORS	11
TOTAL PATIENTS IN PRIMARY EFFICACY ANALYSIS (ITT ANALYSIS)	57
TOTAL PATIENTS IN PRIMARY EFFICACY ANALYSIS (PER PROTOCOL ANALYSIS)	46
TOTAL PATIENTS IN SAFETY ANALYSIS	57

TABLE 3 - PATIENTS WITHDRAWN FROM THE STUDY

	CHOLINE ALPH.
PATIENTS ENROLLED	57
PATIENTS WITHDRAWN	11 *
REASONS:	
LOST TO FOLLOW UP	1*
PROTOCOL DEVIATION	11*
LOW COMPLIANCE	1*

* One patient was a protocol violator, was lost to follow-up and was also a low compliant.

8.2 Protocol Violations

The event classified as protocol deviations were schooling less than five years in 10 patients and poor physical conditions in 1 patient (the same who was also lost to follow-up and had a low compliance to the treatment).

8.3 Baseline Demographic Characteristics

Fourteen males and forty three females were enrolled in the study.

The mean age was 72.1 ± 6.3 (60-80) years, the mean height was 154.5 ±7.2 (141-178) cm and the mean weight was 60.8± 12.5 (36-98) Kg, while the race of the patients was mostly hispanic (n=53); the remaining 4 patients were of a non specified race.

Twenty eight patients had 45 concomitant diseases at baseline: cardiovascular (11 patients), musculoskeletal (7 patients), metabolic (6 patients), gastrointestinal (5 patients), genitourinary (5 patients), CNS (4 patients), respiratory (2 patients), peripheral vascular (2 patients), body as whole (2 patients), dermatological (1 patient).

The concomitant diseases, particularly the CNS ones, did not constitute violation of any of the inclusion criteria of the study protocol.

The neurological examination did not show the presence of any neurological symptoms.

The baseline results of the physical and neurological examination are reported in Table 4.

Table 4 - Summary of physical examination (all patients)

Head, Ears, Eyes, Nose, Throat	Normal	n (%)	36	(63,2)
	Abnormal	n (%)	21	(36,8)
Neck	Normal	n (%)	56	(98,2)
	Abnormal	n (%)	1	(1,8)
Heart	Normal	n (%)	47	(82,5)
	Abnormal	n (%)	10	(17,5)
Respiratory	Normal	n (%)	55	(96,5)
	Abnormal	n (%)	2	(3,5)
Musculoskeletal	Normal	n (%)	34	(59,6)
	Abnormal	n (%)	23	(40,4)
Gastrointestinal	Normal	n (%)	50	(87,7)
	Abnormal	n (%)	7	(12,3)
Peripheral Vascular	Normal	n (%)	40	(70,2)
	Abnormal	n (%)	17	(29,8)
Endocrine/Lymphatic	Normal	n (%)	50	(87,7)
	Abnormal	n (%)	7	(12,3)
Genitourinary	Normal	n (%)	54	(94,7)
	Abnormal	n (%)	3	(5,3)
Dermatological	Normal	n (%)	42	(73,7)
	Abnormal	n (%)	15	(26,3)
CNS	Normal	n (%)	57	(100,0)
	Abnormal	n (%)	0	(0,0)

8.4 Baseline Social Conditions

Table 5 shows the social baseline conditions of the patients enrolled in the study.

TABLE 5 – SOCIAL BASELINE CONDITIONS

Patient Status	Inpatient	0
	Outpatient	57
Marital Status	Single	5
	Married	27
	Separated	3
	Divorced	2
	Widowed	20
	Missing	0
Previous Occupation		
	Top manager	2
	Manager	8
	Executive	12
	Workman	25
	Other	10
Education		
	Degree	7
	High School	11
	Primary School	29
	1-6 years	10
	No schooling	0

CONT. TABLE 5 – SOCIAL BASELINE CONDITIONS

Children	1	3
	2	5
	3	10
	4	10
	5	7
	>5	19
	Missing	3
Siblings	1	5
	2	7
	3	5
	4	9
	5	9
	>5	18
	Missing	4
Twins	Yes	4
	No	51
	Missing	2
Residential Setting	Urban	54
	Suburban	1
	Rural	2
	Missing	0

CONT. TABLE 5 – SOCIAL BASELINE CONDITIONS

Household Income Status (A)	
Dependent	12
Marginal	4
Adequate	40
Missing	1
Household Income Status (B)	
Pension	11
Salary	3
Capital Assets	1
Social Agency	3
Other	32
Missing	7
N. Supported by Income	
1	10
2	15
3	16
4	5
5	3
>5	1
Missing	7

8.5 Baseline Psychometric Evaluations

The baseline scores of the rating scales evaluating the severity of symptoms of Dementia of the Alzheimer Type, depression and cerebrovascular disease are shown in Table 6. According to these scores, in the patients enrolled in the study, the severity of cognitive and behavioural symptoms was mild to moderate.

Table 6 - Baseline Psycometric Evaluations (all patients)

			n	mean	SD	median	min	max
Mini Mental State Examination	visit	-1	57	17,53	3,93	17	12	26
Reisemberg Global Deterioration Scale	visit	-1	57	4,00	0,73	4	3	5
Alzheimer Disease Assessment Scale - cog	visit	0	57	40,12	8,84	39	14	64
Alzheimer Disease Assessment Scale - behavioral	visit	0	57	20,26	6,25	20	10	35
Alzheimer Disease Assessment Scale - total	visit	0	57	60,39	12,37	62	25	93
Clinical Global Impression	visit	0	57	4,09	0,66	4	3	5
Hamilton Depression Scale	visit	0	57	12,46	3,82	13	5	19
Modified Hachinsky Ischemic Scale	visit	0	57	2,11	1,06	2	0	3

8.6 Baseline Values of Laboratory Parameters.

Baseline values (mean, standard deviation, range) of Hemoglobin, Hematocrit, White Blood Cells, Erythrocytes, Lymphocytes, Platelets, Vit. B12, T3, T4, Serum Folate, Glucose, BUN, Creatinine, Uric Acid, Alkaline Phosphatase, SGOT, SGPT, Total Bilirubin, Sodium, Potassium and Chloride are presented in Table 7.

In this table, the normal range limits of lab parameters refer to "Carpermor Laboratorio de Referencia Internacional" located in Mexico City, in which about 90% of laboratory determinations were performed (App. 14.1.8).

Baseline lab data of each Center were classified as "high", "normal" and "low" according to the value of the range limits of its own lab (App. 14.3.3); when abnormal laboratory data were due to haemolysis or technical problems, they were not considered in the analysed data set.

Outlying lab data are considered the clinically relevant ones (i.e. anemia) or relevant to inclusion and exclusion criteria; 169 outlying baseline lab data were collected in 55 patients (App.14.3.3). Most of outlying laboratory data involved electrolytes and hematological parameters. These outlying results were not related to concomitant diseases to be considered as exclusion criteria; therefore the patients were included in the Per Protocol analysis for the primary endpoint of efficacy.

TABLE 7 – BASELINE LABORATORY PARAMETERS

(Population: 57 patients)

PARAMETER	
Hemoglobin (n. v.: 12-18 g/dL)	
No.	54
MEAN	14.41
STANDARD DEVIATION	1.62
MIN-MAX	9.5 – 17.3
Hematocrit (n.v.:37-54%)	
No.	53
MEAN	44.23
STANDARD DEVIATION	4.86
MIN-MAX	32 – 55.7
Total WBC (n.v.: 4.5-10 x1000/ mm^3)	
No.	57
MEAN	5.9
STANDARD DEVIATION	1.53
MIN-MAX	3.05 – 10.5
Total RBC (n.v.: 4.5 –5.8 x mil./mm^3)	
No.	52
MEAN	4.72
STANDARD DEVIATION	0.47
MIN-MAX	3.32 – 5.76
Lymphocytes (n.v.:21-48%)	
No.	57
MEAN	28.47
STANDARD DEVIATION	8.26
MIN-MAX	10.6 – 45.9

CONT. TABLE 7 - BASELINE LABORATORY PARAMETERS

(Population: 57 patients)

PARAMETER	
Platelets (n. v.: 150 – 500 x 1000/ mm^3)	
No.	46
MEAN	234.67
STANDARD DEVIATION	70.47
MIN-MAX	125-465
Vit. B12 (n.v.: 199 – 1526 pg/ml)	
No.	48
MEAN	674.96
STANDARD DEVIATION	336.23
MIN-MAX	255-1610
T3 (n.v.: 0.9 - 2.4 ng/ml)	
No.	57
MEAN	1.46
STANDARD DEVIATION	0.37
MIN-MAX	0.63 – 3.10
T4 (n.v.: 7 - 14.2 Ug/dl)	
No.	56
MEAN	8.41
STANDARD DEVIATION	1.61
MIN-MAX	4.4 – 14.2
Serum Folate (n.v.: 3 – 20 ng/ml)	
No.	53
MEAN	11.27
STANDARD DEVIATION	4.8
MIN-MAX	4.7 – 22.5

CONT. TABLE 7 - BASELINE LABORATORY PARAMETERS

(Population: 57 patients)

PARAMETER	
Glucose (n. v.: 65 – 110 mg/dL)	
No.	56
MEAN	103.16
STANDARD DEVIATION	61.56
MIN-MAX	55 – 471
BUN (n. v.: 10 – 50 mg/dL)	
No.	53
MEAN	35.16
STANDARD DEVIATION	10.31
MIN-MAX	14 – 65
Creatinine (n.v.: 0.5 – 1.2 mg/dL)	
No.	53
MEAN	0.97
STANDARD DEVIATION	0.16
MIN-MAX	0.7 – 1.5
Uric Acid (n.v.: 2.5 – 7 mg/dL)	
No.	55
MEAN	5.23
STANDARD DEVIATION	1.65
MIN-MAX	2.7 – 10.4
Alkaline Phophatase (n.v.: 39–117 U/L)	
No.	56
MEAN	86.63
STANDARD DEVIATION	39.98
MIN-MAX	41 – 297

CONT. TABLE 7 - BASELINE LABORATORY PARAMETERS

(Population: 57 patients)

PARAMETER	
SGOT (n. v.: 5 - 40 U/L)	
No.	55
MEAN	23.64
STANDARD DEVIATION	8.86
MIN-MAX	11 – 52
SGPT (n. v.: 10 – 50 U/L)	
No.	53
MEAN	23.43
STANDARD DEVIATION	11.15
MIN-MAX	6 – 76
Total Bilirubin (n.v.: 0.2 - 1 mg/dL)	
No.	56
MEAN	0.83
STANDARD DEVIATION	1.61
MIN-MAX	0.19 –1.24
Sodium (n.v.: 135 – 145 mEq/L)	
No.	56
MEAN	140.11
STANDARD DEVIATION	3.77
MIN-MAX	132 – 156
Potassium (n.v.: 3.6 - 5 mEq/l)	
No.	49
MEAN	4.52
STANDARD DEVIATION	0.66
MIN-MAX	3.2 –5.7

CONT. TABLE 7 - BASELINE LABORATORY PARAMETERS

(Population: 57 patients)

PARAMETER	
Chloride (n. v.: 98 – 107 mEq/L)	
No.	56
MEAN	103.84
STANDARD DEVIATION	3.86
MIN-MAX	95 – 112

8.7 Baseline Peripheral Hemodynamic Parameters

The baseline values of systolic and diastolic blood pressure (mm Hg) and heart rate (beats per minute) of the patients are shown in Table 8.

TABLE 8 - BASELINE PERIPHERAL HEMODYNAMIC PARAMETERS

Systolic Blood Pressure (mm Hg)		
	Mean	124.7
	St. Dev.	14.7
	Minimum	100
	Maximum	180
Diastolic Blood Pressure (mm Hg)		
	Mean	76.2
	St. Dev.	6.8
	Minimum	60
	Maximum	90
Heart Rate (b.p.m.)		
	Mean	70.8
	St. Dev.	9.5
	Minimum	50
	Maximum	100

9. STUDY MEDICATION

9.1 Dosage and Patient Exposure

As described above, the dosage of the drug was one i.m. vial daily, which corresponds to 1,000 mg choline alphoscerate, during the first three months, and 3 capsules daily, which corresponds to 1,200 mg/day of choline alphoscerate, in the following three months

The administered daily number of vials and capsules was the same for all the treated patients.

9.2 Concomitant Medications

Twenty-eight patients were taking 56 concomitant medications at baseline or at any of the study visits.

Concomitant medications included: NSAIDs in 12 patients, anti-hypertensive drugs in 12 patients, antibiotics in 8 patients, gastrokinetics in 5 patients, oral hypoglycemic agents in 5 patients, CNS active drugs in 4 patients, metabolic drugs in 4 patients, peripheral vascular drugs in 2 patients, others in 4 patients (App. 14.2).

10. EFFICACY RESULTS

10.1 Primary Efficacy End Point - ADAS Cog. Score

ADAS Cog. Score raw mean decreased after 90 days with respect to baseline score, and this decrease continued during the following 90 days of oral treatment (Table 9).

Mean decrease in ADAS Cog. Score after 90 days of i.m. treatment in comparison with baseline score was 6.82 points, while at the end of the study, after 3 months of oral treatment, it was 9.83 points.

Table 10 reports the estimated ADAS Cog. Score contrast obtained as results from the Mixed Linear Model Analysis within treatments.

A statistically significant decrease in ADAS Cog. Score ($p<0.0001$) with respect to baseline scoring was observed after 90 and 180 days of treatment.

No statistically significant differences in ADAS Cog. score were observed between the end of the i.m. treatment and the end of the oral treatment.

Table 9 - Primary endpoint - ADAS Cog.

		n	mean	SD	median	min	max
Day 0 baseline	visit 0	57	40,12	8,84	39	14	64
Day 90 end of i.m.treatment	visit 90	56	33,30	11,58	37	9	59
Day 180 end of oral treatment	visit 180	56	30,29	9,14	28,5	10	59

Table 10 - Summary of efficacy results (ITT and Per Protocol)

	Contrast	Estimate	St. err.	p value	adj p value
ADAS - Cog (ITT)	visit 90 vs. visit 0	-6,97	1,076	<.0001	<.0001
	visit 180 vs. visit 0	-9,93	1,142	<.0001	<.0001
	visit 180 vs. visit 90	-2,96	1,255	0,022	0,0710
ADAS - Cog (PP)	visit 90 vs. visit 0	-7,02	1,242	<.0001	<.0001
	visit 180 vs. visit 0	-10,22	1,178	<.0001	<.0001
	visit 180 vs. visit 90	-3,20	1,342	0,022	0,069

10.2 Secondary Efficacy End Points – Psychometric Evaluations

Table 11 shows raw means and other computed parameters of MMSE, GDS, ADAS Behav. and Total and CGI.

MMSE score increased by 4.31 points after 90 days and by 6.01 points after 180 days with respect to baseline score.

GDS stage decreased by 0.7 points after 90 days and by 1.07 points after 180 days with respect to baseline score.

ADAS Behav. score decreased by 3.63 points after 90 days and by 4.15 points after 180 days with respect to baseline score.

ADAS Total score decreased by 10.46 points after 90 and by 14 points after 180 days with respect to baseline score.

CGI score decreased by 0.43 points after 90 days and by 0.96 points after 180 days with respect to baseline score.

Table 12 reports the estimated MMSE, GDS, ADAS Behav. and Total and CGI Score contrast obtained as results from the mixed Linear Model Analysis within treatments using ITT population.

A statistically significant changes in MMSE, GDS, ADAS Behav and Total and CGI scores ($p< 0.0001$) with respect to baseline scoring was observed after 90 and 180 days.

Statistically significant differences in MMSE ($p=0.01$), GDS ($p=0.0002$) and CGI ($p<0.0001$) results were observed between the end of the i.m. treatment and the end of the oral treatment.

Table 11 - Psychometric evaluations - Secondary Endpoints

		n	mean	SD	median	min	max
MMSE	visit -1	57	17,53	3,93	17	12	26
	visit 90	56	21,84	3,99	22	12	30
	visit 180	56	23,54	4,39	24,5	8	29
GDS	visit -1	57	4,00	0,73	4	3	5
	visit 90	56	3,30	0,66	3	2	5
	visit 180	56	2,93	0,68	3	2	4
ADAS Behav	visit 0	57	20,26	6,25	20	10	35
	visit 90	56	16,63	3,47	16	10	27
	visit 180	56	16,11	2,83	16	10	27
ADAS Total	visit 0	57	60,39	12,37	62	25	93
	visit 90	56	49,93	13,81	53,5	23	83
	visit 180	56	46,39	11,43	45	21	80
Clinical Global Impression	visit 0	57	4,09	0,66	4	3	5
	visit 90	56	3,66	0,67	4	3	5
	visit 180	56	3,13	0,72	3	2	5

Table 12 - Summary of efficacy results - model mixed contrasts (all patients)

	Contrast	Estimate	St. err.	p value	adj p val. †
MMSE	visit 90 vs. visit -1	4,42	0,379	<.0001	<.0001
	visit 180 vs. visit -1	6,07	0,626	<.0001	<.0001
	visit 180 vs. visit 90	1,65	0,526	0,0027	0,0108
GDS (Score)	visit 90 vs. visit -1	-0,70	0,091	<.0001	<.0001
	visit 180 vs. visit -1	-1,08	0,093	<.0001	<.0001
	visit 180 vs. visit 90	-0,38	0,083	<.0001	0,0002
ADAS Behav	visit 90 vs. visit 0	-3,66	0,827	<.0001	0,0002
	visit 180 vs. visit 0	-4,17	0,832	<.0001	<.0001
	visit 180 vs. visit 90	-0,51	0,414	0,2239	0,4741
ADAS Total	visit 90 vs. visit 0	-10,68	1,495	<.0001	<.0001
	visit 180 vs. visit 0	-14,12	1,701	<.0001	<.0001
	visit 180 vs. visit 90	-3,44	1,482	0,0241	0,0770
CGI (Score)	visit 90 vs. visit 0	-0,44	0,088	<.0001	<.0001
	visit 180 vs. visit 0	-0,97	0,097	<.0001	<.0001
	visit 180 vs. visit 90	-0,54	0,084	<.0001	<.0001
Global Improvement	visit 180 vs. visit 90	-0,30	0,130	0,0229	0,0229
GDS (Ranks)	visit 90 vs. visit -1	-38,62	5,147	<.0001	<.0001
	visit 180 vs. visit -1	-58,93	5,222	<.0001	<.0001
	visit 180 vs. visit 90	-20,31	4,753	<.0001	0,0004
CGI (Ranks)	visit 90 vs. visit 0	-26,41	5,295	<.0001	<.0001
	visit 180 vs. visit 0	-56,56	5,552	<.0001	<.0001
	visit 180 vs. visit 90	-30,15	5,018	<.0001	<.0001
Global Improvement (Ranks)	visit 180 vs. visit 90	-12,77	3,147	0,0002	0,0002

† p value adjusted using Scheffé approach for multiple comparisons

10.3 Other Efficacy Results

10.3.1 Global Improvement

Global Improvement score was 2.21 ±1.01 at day 90 and 1.9 ±1.04 at day 180 with an decrease by 0.31 points after 180 days with respect to day 90.

These results indicate that the severity of the disease was reduced after 90 days of i. m. treatment, and that this reduction continued during oral treatment.

A statistically significant difference ($p<0.02$) was observed between the end of the i.m. treatment and the end of the oral treatment.

10.3.2 Patients Termination Records

Table 13 summarizes the characteristics of the patients at the end of the study period.

99.2% of patients completed the study and took the drug as prescribed or without relevant irregularities (In patients "completing" the study are included patients in which drug was discontinued by Investigators due to adverse events, see Par. 11.1)

37 out of 57 patients experienced above average (27 patients) or far above average (10 patients) clinical improvement at the end of i.m. treatment period.

39 out of 57 patients experienced above average (28 patients) or far above average (11 patients) clinical improvement at the end of oral treatment period.

When patients were asked if they wished to continue the i.m. treatment with the study drug, 54 patients gave positive answers: 13 patients were inclined to say yes and 41 patients said definitively yes.

When patients were asked if they wished to continue the oral treatment with the study drug, 50 patients gave positive answers: 9 patients were inclined to say yes and 41 patients said definitively yes.

Table 13 **Patients Termination Records**

			Visit			
			Day 90 - I.M.		Day 180 - Os	
		Statistic	N = 57		N = 57	
Days on study						
	0	n (%)	1	(1,8)	1	(1,8)
	73	n (%)	0	(0,0)	1	(1,8)
	87	n (%)	1	(1,8)	0	(0,0)
	90	n (%)	55	(96,5)	55	(96,5)
Premature termination						
	No premature termination	n (%)	55	(96,5)	54	(94,7)
	Improvement	n (%)	1	(1,8)	1	(1,8)
	Intercurrent Illness	n (%)	0	(0,0)	1	(1,8)
	Found not meet study criteria	n (%)	1	(1,8)	1	(1,8)
Interval history (1)						
	Missing	n (%)	1	(1,8)	1	(1,8)
	0	n (%)	42	(73,7)	39	(68,4)
	1	n (%)	3	(5,3)	2	(3,5)
	2	n (%)	2	(3,5)	1	(1,8)
	3	n (%)	0	(0,0)	4	(7,0)
	4	n (%)	3	(5,3)	6	(10,5)
	5	n (%)	1	(1,8)	4	(7,0)
	6	n (%)	3	(5,3)	0	(0,0)
	7	n (%)	1	(1,8)	0	(0,0)
	8	n (%)	1	(1,8)	0	(0,0)
Drug Intake						
	Missing	n (%)	0	(0,0)	1	(1,8)
	Not receive drug	n (%)	1	(1,8)	0	(0,0)
	Took treatment as prescribed	n (%)	50	(87,7)	52	(91,2)
	Not significant irregularities	n (%)	5	(8,8)	4	(7,0)
	Suspect significant irregularities	n (%)	1	(1,8)	0	(0,0)
Disposition at termination - Outpatients (2)						
	Missing	n (%)	0	(0,0)	1	(1,8)
	2	n (%)	0	(0,0)	1	(1,8)
	3	n (%)	54	(94,7)	45	(78,9)
	4	n (%)	0	(0,0)	1	(1,8)
	5	n (%)	1	(1,8)	8	(14,0)
	6	n (%)	2	(3,5)	1	(1,8)

1) 0= no significant events or changes 1=catastrophic event 2=death of significant other 3=physical/mental illness of significant other 4= difficulties in relationship with relatives or peers 5=decrease in status and/or responsability 6=improvement in relationship with relatives or peers 7=increase in staus and/or responsability 8=Pregnancy of subject (spouse or parents) and or birth of child/sibling.

2) 0=discharged against medical advice 1=hospitalized (transferred to inpatient status) 2=remains on outpatient status and treatment is intensified because of exacerbation 3=remains on outpatient status and status is unchanged 4= remains on outpatient status and treatment is reduced because of improvement 5=discharged to own custody or own family 6=transferred or discharged for reasons unrelated to present

Cont. Table 13 **Patients Termination Records**

			Visit				
			Day 90		Day 180		
		Statistic	N = 57		N = 57		p value ‡
Study drug continuation							
	Missing	n (%)	1	(1,8)	1	(1,8)	0,499
	Definitely no	n (%)	0	(0,0)	2	(3,5)	
	Inclined to say no	n (%)	2	(3,5)	4	(7,0)	
	Undecided	n (%)	0	(0,0)	0	(0,0)	
	Inclined to say yes	n (%)	13	(22,8)	9	(15,8)	
	Definitely yes	n (%)	41	(71,9)	41	(71,9)	

‡ Cochran Mantel Haenszel

11. SAFETY RESULTS

The safety population included all patients who had at least one safety evaluation after administration of choline alphoscerate. 57 patients were included in the safety analysis.

11.1 Overall Experience of Adverse Events.

Overall, fifty-one adverse events were reported in 21 patients: 26 in 16 patients with i.m. vials and 25 in 17 patients with oral capsules (12 patients reported adverse events during both i.m. and oral treatment).

Out of these 51 adverse events, no one was classified as serious.

Adverse events classified by body system and administered formulation is presented in Table 14 and App.14.3.4.

Event severity, action taken, outcome and relationship to study drug is presented in Table 15 and App. 14.3.4.

The frequency, type and severity of the recorded adverse events were similar during both i.m. vials and oral capsules treatments.

Most of adverse events involved CNS or digestive system; their severity was mild or moderate in 50 of the 51 patients.

Adverse reactions (i.e. adverse events for which the causal relationship between study drug and event was stated by the investigator as highly probable, probable or possible) are presented in Table 16 and App.14.3.4.

Overall, 29 adverse drug reactions were reported, in 18 patients: 15 in 11 patients with i.m. vials and 14 in 9 patients with oral capsules (2 patients reported adverse reactions during both i.m. and oral treatment); in most of patients, adverse drug reactions were mild.

TABLE 14 – ADVERSE EVENTS BY BODY SYSTEM
Population: 57 patients

		Number of adverse events (No. AE)		
Body System	**Description**	CHOLINE ALPHOSCERATE		
		I.M *	**OS ****	**TOTAL**
Body as a Whole	Chest Pain	1	0	1
	Injection site pain	1	0	1
	Carcinoma (skin)	0	1	0
	Ear pain	0	1	1
Digestive System	Gastritis	0	1	1
	Gastric Disease	0	1	1
	Constipation	1	0	1
	Diarrhoea	1	0	1
	Dry Mouth	1	0	1
Nervous System	Somnolence	4	3	7
	Insomnia	4	3	7
	Hostility	2	3	5
	Anxiety	2	0	2
	Nervousness	1	2	3
	Cerebral ischemia	1	2	3
	Brain injury	1	0	1
	Migraine	1	0	1
	Cerebral hematoma	1	0	1
	Personality Disorders	0	1	1
	Hallucinations	0	1	1
	Convulsion	0	1	1
	Hyperkinesia	0	1	1
	Hypertonia	0	1	1
Skin and Appendages	Urticaria	1	0	1
Urogenital System	Cystitis	1	0	1
	Urinary Infection	0	1	1
Cardiovascular System	Extrasystols	1	0	1
	Varicous Veins	1	1	2
	Arrhytmia	0	1	1
Platelet, bleeding Disorders	Thrombocitopenia	0	1	1
Total Adverse Events		**26**	**25**	**51**

* Recorded in 16 patients
** Recorded in 17 patients

TABLE 15 – ADVERSE EVENTS DESCRIPTION Population: 57 patients			
		LM *	OS **
Severity	Mild Moderate Severe	14 12 0	14 10 1
Action Taken	None Counteractive Medication Drug Discontinued Permanently Dose Reduction Other	10 6 10*** 0 0	4 7 1*** 1 12
Outcome	Recovery Improvement Insufficient Follow-Up	19 7 0	12 12 1
Relationship	Highly Probable Probable Possible Remote None	8 7 0 0 11	3 11 0 1 10

* Recorded in 16 patients
** Recorded in 17 patients
*** None of these events was related to study drug administration

TABLE 16 – ADVERSE DRUG REACTIONS BY BODY SYSTEM Population: 57 patients				
		Number of adverse Drug reactions (No. ADR)		
Body System	Description	CHOLINE ALPHOSCERATE		
		I.M *	OS **	TOTAL
Body as a whole	Injection site pain	1	0	1
Digestive System	Dry Mouth Gastritis Gastric Disease	1 0 0	0 1 1	1 1 1
Nervous System	Somnolence Insomnia Hostility Nervousness Anxiety Convulsions Hyperkinesia	4 4 2 1 1 0 0	3 2 3 2 0 1 1	7 6 5 3 1 1 1
Skin and Appendages	Urticaria	1	0	1
Total ADR		**15**	**14**	**29**

* Recorded in 11 patients
** Recorded in 9 patients

11.2 Adverse Drug Reactions Leading to Discontinuation

No adverse drug reactions leading to discontinuation of choline alphoscerate were recorded during the study period.

11.3 Other Safety Results

11.3.1 Investigator's Rating of Tolerability

Table 17 shows the frequency and severity of ratings. The most frequent rating was " Good" both for vials (47.3 %) and capsules (50.9 %).

The results indicate that in the opinion of the Investigators choline alphoscerate was safe and well tolerated.

Table 17 - Investigators Evaluation of Choline Alphoscerate Tolerability

	DAY 90 – I.M.		DAY 180 - OS	
	Frequency	%	Frequency	%
Very Good	20	35.1 %	23	40.4 %
Good	27	47.3 %	29	50.9 %
Acceptable	8	14 %	2	3.5 %
Poor	1	1.8 %	2	3.5 %
Missing	1	1.8 %	1	1.7 %
Total	57	100%	57	100%

12. DISCUSSION AND OVERALL CONCLUSIONS

The positive efficacy results gained in this open, uncontrolled study are to be cautiously evaluated, as they were not be properly compared with a no-treatment situation.
Nevertheless, two different points can be suggested, to give some relevance to the efficacy results of this study:

- The study results could be evaluated in comparison with published longitudinal studies in non treated SDAT patients, in which the natural worsening of SDAT over the time is described (37);
- anyway, the efficacy results of this study could be regarded as "confirmatory" of the clinical results gained in previous published trials with choline alphoscerate (25-34), and in particular observed in Study COL/95/2: Double blind, placebo controlled study in mild to moderate SDAT patients (38) recently completed by the same Investigators of this trial.

According to the data on treatment side effects collected in this study, both the administered formulations had a good profile of tolerability, and can be considered safe and suitable for a long-term treatment. In particular it must be stressed that, as for incidence, type and severity of adverse reactions, the clinical tolerability of the i.m. formulation of choline alphoscerate resulted comparable to that of the oral form.

In conclusion, the results of this open, multicenter, non controlled trial confirm that choline alphoscerate i.m vials and oral capsules are safe and effective in the long term treatment of mild to moderate Dementia of the Alzheimer Type.

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14. APPENDICES

14.1 Study information

14.1.1 Protocol

14.1.2 Informed Consent

14.1.3 Protocol Amendment (Amendment No.1)

14.1.4 Sample case report form

14.1.5 List of Institutional Review Boards

14.1.6 List of Investigators, Co-investigators and Centers

14.1.7 Randomization scheme and codes (not implemented, following Amendment n. 1)

14.1.8 Laboratories Quality Standards and Normal Ranges

14.1.9 Audit Certificates

14.2 Statistical Report

14.3 Patient data listings

14.3.1 Discontinued patients

14.3.2 Protocol deviations/violations

14.3.3 Classification of lab data into "low", "normal" and "high"; outlying lab data

14.3.4 Adverse events/Serious adverse event listing

14.3.5 Individual patient data listing