

ApoE Genotyping and Response to Galanthamine in Alzheimer's Disease – A Real Life Retrospective Study

Tomislav Babić¹, Darija Mahović Lakušić¹, Jadranka Sertić²,
Mladen Petrovečki³ and Ana Stavljenić-Rukavina²

¹ Department of Neurology, University Hospital Center »Zagreb«, Zagreb, Croatia

² Department of Laboratory Medicine, School of Medicine, University of Zagreb, Zagreb, Croatia

³ Department of Laboratory Medicine, University Hospital »Dubrava«, Zagreb, Croatia

ABSTRACT

This study was undertaken to evaluate the effect of galanthamine, a new cholinesterase inhibitor on cognitive performances in 84 patients with various apoE genotype and Alzheimer's disease (AD) during the six-month treatment. The diagnosis of AD was made on the basis of NINCDS/ADRDN criteria. ApoE4 genotype was determined by PCR procedure. The cognitive performance was assessed MMSE at baseline and six months later. The difference among the groups was statistically analyzed by ANOVA model and Pearson's χ^2 -test. The MMSE at baseline in all completes was 18.0 ± 3.73 , whereas the mean value of MMSE after 6 months was 16.4 ± 5.61 indicating significant deterioration ($p < 0.01$). Of the 84 patients, 14 (16%) were apoE4 homozygous, 41 (49%) were heterozygous, whereas 29 (35%) were apoE4 negative. The significant number of responders was observed among apoE4 homozygous patients (71%; $\chi^2 = 6.89$; $p = 0.032$). The subgroup of apoE4 homozygous patients with AD in its mild to moderate stage may be considered as responders to galanthamine.

Introduction

Alzheimer's disease (AD) is progressive degenerative disorder, characterized by deficits in memory and cognition that are associated with significant losses of presynaptic cholinergic function in the brain¹. It has been speculated that cholinomimetics might improve cognitive

performances in patients with AD². Galanthamine, a reversible acetylcholinesterase (AChE) inhibitor that can be isolated from a number of different plant sources, including daffodil bulbs, has been used mainly in Eastern Europe as an antagonist of non-depolarizing muscle rela-

xants³. Animal studies indicate that galanthamine can improve learning and memory performances⁴. Several clinical studies have recently shown that galanthamine might be useful in the treatment of cognitive decline in AD^{5,6}, but there are no biological markers which can predict therapeutic response. Apolipoprotein E4 (apoE4) is a well known risk factor for the onset of AD⁷. However, it seems that the presence of apoE4 alleles have a negative therapeutic effect in AD patients treated by tacrine⁸.

The present study was undertaken to evaluate the efficacy and tolerability of galanthamine at a dosage level of 30 mg/day during six month treatment of patients with various apoE genotypes who suffered from mild to moderate AD.

Patients and Methods

Patients

Patients eligible for this study were newly diagnosed patients of uncomplicated, probable AD. The diagnosis of probable AD was made according to the criteria outlined by National Institute of Neurological and Communicative Disorders and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA criteria)⁹ with no clinical or laboratory evidence of a cause other than AD for their dementia. Patients had scores on the Mini-Mental State Examination (MMSE)¹⁰ 12–24, at both screening and baseline. Patients with non-Alzheimer's dementia, severe Alzheimer's dementia (MMSE < 12), and patients with Alzheimer's dementia complicated by psychosis and significant dysphasia were excluded from the study. In addition, patients with concomitant diseases (chronic obstructive airway disease, bradycardia, heart block, active peptic ulcer, prostate disease) or patients with concomitant medication which might interfere with the effect of galanthamine (anticholinergics, antide-

pressants, anxiolytics other than short acting benzodiazepines for insomnia, antiparkinsonics, NSAID, antiepileptics, cimetidine) were excluded from the study prior to baseline. Patients who were known to be hypersensitive to AChE inhibitors were excluded. Patients were required to have reliable caregiver. Written informed consent was obtained from both the patient and from their caregiver.

Study design

This was a patient's name basis, 6-month open label study. The trial was conducted on outpatient basis. One hundred and twenty-six patients were enrolled in the therapeutic study. Following 2–4 weeks of screening period, initially one galanthamine hydrobromide tablet of 5 milligrams was administered daily. A daily dose of galanthamine was increased by 5 mg weekly, until optimal therapeutic dose of 30 mg/day (two tablets) was achieved. Patients who took less than 30 mg of galanthamine daily more than 14 days during the study were excluded from the analysis.

Outcome measure was Mini Mental Status Examination (MMSE) and it was carried out by an independent neurologist who was not aware of the patient's ApoE status before the treatment and at 6 months. Safety was assessed by physical examination, clinical laboratory tests, adverse event monitoring, and evaluation of the general health and well-being of the patient every two weeks in the first three-month period, then every four weeks thereafter. Compliance was assessed every four weeks, counting the tablets returned.

Blood for ApoE genotypization was taken during the screening phase and was analyzed from frozen samples by polymerase chain reaction (PCR) procedure described by Hixon and Vernier¹¹.

A statistical analysis was done in all patients who completed a trial according

to protocol. (Completes). Completes were defined as patients who completed 6 months of treatment with at least 80% compliance of study medication at month 6. ANOVA model was used for quantitative analysis of efficacy variable. Qualitative analysis (responders' rate) among the groups was done by Pearson's χ^2 -test. Responders were defined as completes who did not deteriorate at the end of month 6.

Results

From 126 patients who started with galanthamine treatment 84 (69%) patients completed 6 month treatment period. The baseline characteristics of all patients are shown in Table 1. In all groups, there was an approximately equal proportion of men and women. The distribution of mean age, duration of Alzheimer's disease, a frequency of familiar history of

TABLE 1
BASELINE AND END-POINT CHARACTERISTICS OF APOE SUBGROUPS OF PATIENTS

Parameter	All	ApoE4 heterozygous	ApoE4 homozygous	ApoE4 negatives
All patients				
Number of patients: N (%)	126	62 (50.2%)	21 (16.7%)	43 (34.1%)
Age: X±SD (range)	68.5 ± 7.2 (51–89)	69.1 ± 6.9 (51–84)	70.7 ± 7.7 (51–85)	67.2 ± 7.4 (56–88)
Sex (M/F)	65/61	35/27	9/12	22/21
Duration of AD (years): X±SD (range)	3.0 ± 1.7 (1.2–8.0)	3.0 ± 1.6 (1.7–7.1)	3.2 ± 1.9 (1.1–6.4)	2.8 ± 1.8 (1.4–6.8)
Familial history of AD (N)	25	13	6	6
Baseline MMSE: X±SD (range)	18.0 ± 3.52 (12–24)	17.9 ± 3.54 (12–24)	18.1 ± 3.41 (12–23)	18.2 ± 3.58 (12–24)
Completes only				
Number of patients (N)	84	41 (49%)	14 (16%)	29 (35%)
Age: X±SD (range)	68.9 ± 7.1 (51–87)	69.4 ± 8.3 (53–84)	71.1 ± 6.7 (51–85)	67 ± 7.4 (57–87)
Sex (M/F)	42/42	23/18	5/9	14/15
Duration of AD (years): X±SD (range)	3.1 ± 1.8 (1.2–7.1)	3.0 ± 1.6 (1.7–7.1)	3.1 ± 2.1 (1.2–6.3)	3.0 ± 1.9 (1.8–6.6)
Familial history of AD (N)	17	9	3	5
Baseline MMSE: X±SD (range)	18.0 ± 3.73 (12–24)	17.7 ± 3.76 (12–24)	18.2 ± 3.14 (12–23)	18.2 ± 4.04 (13–24)
End-point MMSE: X±SD (range)	16.4 ± 5.61** (5–30)	15.9 ± 5.26 (8–27)	18.4 ± 4.43 (10–25)	16.2 ± 6.51 (5–30)
End-point responders (N)	34 (40%)	15 (37%)	10 (71%)	9 (31%)
$(\chi^2 = 6.89; p = 0.032)$				

** p < 0.01 (ANOVA model)

disease, and mean MMSE results did not differ significantly across the three groups. Twenty-nine patients did not complete the study due to adverse events, whereas 13 patients were excluded due to lack of compliance.

55 out of 84 completes (65%) were apoE4 carriers, having at least one apoE4 allele. Fourteen patients (16%) were apoE4 homozygous and 41 (49%) were apoE4 heterozygous. Twenty-nine patients (35%) were apoE4 negative. The frequency of ApoE alleles among completes was $e3 = 0.58$; $e4 = 0.41$; $e2 = 0.01$.

The baseline characteristics of the completes and non-completes were similar, and in all groups, there was no difference in sex, mean age, duration of Alzheimer's disease, a frequency of familial history of disease. The mean MMSE results did not differ significantly as well across the three groups of completes and non-completes.

As indicated in Table 1 the mean MMSE score for all completes deteriorated after six months. The mean value of MMSE at baseline in all completes was 18.0 ± 3.73 (range 12–24), whereas the mean value of MMSE after 6 months was 16.4 ± 5.61 (range 5–30). The deterioration was statistically significant ($p < 0.01$). Among three subgroups of patients, apoE4 negative patients and apoE4 heterozygous patients deteriorated after 6 months of treatment. Only the subgroup of apoE4 homozygous improved slightly after six months of treatment. The percentages of responders were in apoE4 homozygous 71%, apoE heterozygous 37% and apoE negative 31%. The difference in responders rate among the genetic subgroups was significant ($\chi^2 = 6.89$; $p = 0.032$), suggesting that only apoE4 homozygous subgroup of patients with Alzheimer's disease might be considered as responders on galanthamine.

Discussion

This study suggests that 6-month treatment with galanthamine does not improve cognitive performances in patients with mild to moderate AD. However, it seems that apoE4 homozygous patients with AD react favorably to other apoE subgroups, and might be considered as possible responders to galanthamine. This result is not consistent with previously reported effect of tacrine⁸, where apoE4 patients with AD were considered as non-responders to tacrine. We believe that the observed difference may be a clinically important consequence of different type of action of various AChE inhibitors¹² on cognitive performances. Galanthamine acts differently than tacrine in at least two directions. It is a direct nicotinic agonist via different binding sites and independently of the AChE activity¹³. In addition galanthamine is acting as a stimulator of realizing of adrenocorticotrophic hormone (ACTH)¹⁴ that could play role in the anti-inflammatory process in AD. The clinical implication of these pharmacodynamic features of galanthamine on cognitive performances in apoE4 homozygous AD sufferers need to be tested in randomized controlled clinical trials.

At present, galanthamine and other ACE inhibitors as tacrine¹⁵, donepezil¹⁶ and rivastigmine¹⁷ are licensed for the symptomatic treatment of mild to moderate cognitive impairment, but the benefit could be expected in only 20% of patients¹⁸. It has been proposed that treatment of cognitive decline in AD should be at least 6 months long to define responders¹⁹. We feel that the results of our study will help in the early identification of potential responders, thus avoiding unnecessary exposure to AChE inhibitors.

There are several limitations to our study. The open label uncontrolled clinical studies are susceptible to bias, and such results must be analyzed with care.

Furthermore, MMSE may be insensitive to change over short period of time in patients with AD. We wish to point out that our study is preliminary involving a small proportion of ApoE4 homozygous patients. Despite limitation, we believe that our results are sufficiently encouraging

to warrant a larger prospective controlled trial with more sensitive outcome measures like Alzheimer's disease assessment scale (ADAS)²⁰ or Clinical Interview Based on the Impression of Change (CIBIC)²¹.

REFERENCES

1. DAVIES, P., A. J. MALONEY, Lancet, 2 (1976) 1403. — 2. IQBAL, K., H. M. WISNIEWSKI, B. WINDBLAD: Alzheimer's disease and related disorders. (Alan R Liss Inc., New York, 1989). — 3. HARVEY, A. L., Pharmac. Ther., 68 (1995) 113. — 4. SWEENEY, J. E., C. F. HOHMANN, T. H. MORAN, J. T. COYLE, Pharmacology and Behaviour, 31 (1988) 141. — 5. DAL-BIANCO, P., J. MALY, C. WOBER, C. LIND, G. KOCH, J. HUFGARD, I. MARSCHALL, M. MRAZ, L. DEECKE, Neural. Transm., 33 Suppl. (1991) 59. — 6. FULTON, B., P. BENFIELD, Drugs Aging, 9 (1996) 60. — 7. POIRIER, J., J. DAVIGNON, D. BOUTHILLIER, S. KOGAN, P. BERTRAND, S. GAUTHIER, Lancet, 342 (1993) 697. — 8. POIRIER, J., M. C. DELISLE, R. QUIRION, I. AUBERTT, M. FARLOW, D. LAHIRI, S. HUI, P. BERTRAND, J. NALBANTOGLU, B. M. GILFIX, Proc. Natl. Acad. Sci. USA, 92 (1995) 12260. — 9. MCKAHN, G., D. DRACHMAN, M. FOLSTEIN, R. KATZMAN, D. PRICE, E. M. STADLAN, Neurology, 34 (1984) 939. — 10. FOLSTEIN, M., S. FOLSTEIN, P. MCHUGH, J. Psych. Res., 12 (1975) 189. — 11. HIXON, J. E., D. T. WERNIER, J. Lipid. Res., 31 (1990) 545. — 12. THOMSEN, T., B. KADEN, J. P. FISCHER, U. BICKEL, H. BARZ, G. GUSZTONY, J. CERVOS-NAVARRO, H. KEWITZ, Eur. J. Clin. Chem. Clin. Biochem., 29 (1991) 487. — 13. PEREIRA, E. F., M. ALKONDON, S. REINHARDT, A. MAELICKE, X. PENG, J. LINDSTRO, P. WHITING, E. X. ALBUQUERQUE, J. Pharmacol. Exp. Ther., 270 (1994) 768. — 14. COZANTIS, D., A. DESSYPRIS, K. NUUTILA, Acta Aneast. Scand., 28 (1980) 166. — 15. DAVIS, K. L., L. J. THAL, E. R. GAMZU, N. Engl. J. Med., 327 (1992) 1253. — 16. ROGERS, S. L., M. R. FARLOW, R. S. DOODY, R. MOHS, L. T. FRIEDHOFF, Neurology, 50 (1998) 136. — 17. COREY-BLOOM, J., R. ANAND, Int. J. Geriatr. Psychopharmacol., 1 (1998) 55. — 18. LARNER, A. J., S. F. FARMER, BMJ, 319 (1999) 362. — 19. COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP), (The European Agency for the Evaluation of Medicinal Products, London, 1997). — 20. ROSEN, W. G., R. C. MOHS, K. L. DAVIS, Am. J. Psych., 14 (1984) 1356. — 21. LEBER, P., (Food and Drug Administration, Centre for Drug Evaluation and Research, Office of Drug Evaluation, Division of Neuropharmacologic Products, Rockville, 1990).

T. Babić

Department of Neurology, University Hospital Center »Zagreb«, Kišpatićeva 12,
10000 Zagreb, Croatia
e-mail: tomislav.babic@zg.tel.hr

APO-E GENOTIPIZIRANJE I ODGOVOR NA GALANTAMIN U ALZHEIMEROVOJ BOLESTI – RETROSPEKTIVNA STUDIJA

S A Ž E T A K

Svrha ovog istraživanja je bila procijeniti utjecaj galantamina, novog inhibitora kolineraze na kognitivne funkcije u 84 bolesnika s različitim ApoE genotipom i Alzheimerovom bolešću za vrijeme šestomjesečne terapije. Dijagnoza AD postavljena je na osnovu NINCDS/ADRDN kriterija. ApoE4 genotip je određen pomoću PCR postupka. Kognitivne funkcije bolesnika procijenjene su pomoću MMSE na početku istraživanja i nakon šest mjeseci. U statističkoj analizi razlike među skupinama testirane su pomoću ANOVA-e i Pearsonovog χ^2 -testa. Na početku istraživanja MMSE iznosio je $18,0 \pm 3,73$, dok je nakon šest mjeseci MMSE iznosio $16,4 \pm 5,61$ što indicira značajno pogoršanje ($p < 0,01$). Od 84 bolesnika, 14 (16%) bili su ApoE4 homozigoti, 41 (49%) heterozigoti, dok su 29 (35%) bolesnika bili ApoE4 negativni. Značajan broj bolesnika koji su pozitivno reagirali na primjenu galantamina zamijećeno je u skupini ApoE4 homozigotnih bolesnika (71%; $\chi^2 = 6,89$; $p = 0,032$). Rezultati studije sugeriraju pozitivan odgovor na galantamin u skupini bolesnika koji su homozigoti za ApoE4 genotip.