APOLIPOPROTEIN E: A NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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1. INTRODUCTION

Alzheimer disease (AD) is associated with neuronal loss, synaptic damage, deposition of beta-amyloid and loss of cholinergic activity in susceptible brain regions. The ladder three pathological markers of AD were shown to be closely associated with the presence of the apolipoprotein ε4 (apoε4) allele in sporadic AD subjects. The apoε4 allele is a well known risk factor for sporadic late onset Alzheimer's disease; patients with two ε4 alleles exhibit an earlier age of onset, higher amyloid plaque counts, cerebrovascular amyloid, marked reductions in choline acetyltransferase and nerve growth factor receptor density as compared to non-ε4 allele subjects. Recent evidence suggest that apoE polymorphism may significantly affect the clinical presentation of the disease as well as the global efficacy of memory enhancer drugs such acetylcholine esterase inhibitors and noradrenergic modulators. Several different hypotheses have been presented to explain the effect of the ε4 allele on the age of onset and clinical progression of the disease. Because of the reported effect of low levels of apoE on synaptic plasticity, reinnervation and lipid homeostasis in apoE knockout mice, it has been proposed a little while ago that the low levels of apoE reported in brain tissues of apoε4 carriers affect lipid homeostasis in such a way that it compromises synaptic plasticity.[1]

This study was designed to examine the effect of Probucol on the clinical course of mild-to-moderate AD subjects in a six month clinical trial. Probucol is a potent inducer of apoE expression that affects both mRNA and protein concentrations in the brain. The mechanism of the reduction in LDL-cholesterol levels is yet to be fully elucidated but it could results from enhanced catabolism of lipoprotein complexes by lipoprotein lipases. There is evidence of an *independent* antioxidant effect. Probucol has an excellent safety profile with very limited side effects.[2] The ability of Probucol to enhance apoE synthesis and secretion was first demonstrated in primary type 1 astrocyte

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cultures from rodents. The biochemical effect of Probucol on brain apoE metabolism was confirmed in the brain of C57/BL 6J mice after short term intra-peritoneal administration of the active agent. Finally, the drug was used in a 6 month "proof-of-principle" drug trial with patients suffering from mild to moderate Alzheimer's disease.

2. MATERIALS AND METHODS

2.1. Probucol Affects apoE Synthesis and Secretion in Primary Type 1 Astrocyte Cultures and the Brain of C57/Bl 6J Mice

Primary cultures of type 1 astrocytes were derived from the cortex of 1 to 2 day-old Sprague-Dawley rats. Probucol was dissolved is in culture media and added to cell for 24 and 48 hrs. Media was then removed and kept frozen at −80°C until dosage of apoE protein levels by Western blot or apoE mRNA levels by real-time quantitative PCR using a modification of the protocol of Powell and Kroom.[3] C57/BL 6J (Jackson Laboratory, Bar Harbor, USA) mice (n=10) received daily i.p. injections of Probucol dissolved in saline at final concentrations of 0.1, 0.5 and 5 mg/kg. After 10 days of treatment, animals were sacrificed. Tissues samples were kept frozen at -80°C until dosage of apoE.

2.2. Probucol Induces apoE in the Cerebrospinal Fluid and Clinical Outcome in Alzheimer's Disease

Eleven patients with probable AD (NINCD-ADRDA, 1984) were enrolled in a "proof-of -principle" study designed to examine the effect of apoE induction on the clinical course of the disease. All subjects had to have no significant medical problems and not be on any medication that could interfere with their cognitive performance. They had to be between 50 and 80 years of age, to exhibit Mini Mental State Exam (MMSE) score between 10 and 26 and to be fluent in French or English. All patients received Probucol 500 mg twice daily for a period of six months. Lumbar punctures were performed at baseline **and** 1 month after onset of treatment to determine the quantity of CSF apoE, peroxidised lipids, total Tau and beta amyloid 1-40 levels in each subjects. Alzheimer's Disease Assessment Cognitive Scale (ADAS-Cog) and Disability Assessment of Dementia (DAD) scale ratings were performed at baseline, months 3 and 6 to monitor cognitive and global functioning. Blood was obtained at baseline, month 3 and 6 to assay levels of cholesterol and triglycerides.

3. RESULTS

We observed an increased production and secretion of apoE by Probucol in type 1 astrocyte cultures after 24 hours exposure. It returns to near control levels within 48 hours. A concomitant induction of apoE mRNA prevalence was observed in parallel astrocytic cultures at 8, 24 and 48 hrs. Intraperitoneal administration of Probucol was shown to increase steady state levels of apoE in periphery and in the brain. The mid-range dose (0.5 mg/kg/day) was selected to approximate the concentration of Probucol used to lower blood cholesterol in humans. Western blot analyses of apoE levels in mice liver and plasma tissues reveals an inverse bell shape dose response with a peak level at the lowest concentration of Probucol (0.1 mg/kg/day). However, the effect of Probucol is

somewhat different in the central nervous system of these animals. A clear dose-dependent increase in the steady state levels of apoE was observed the hippocampus of these animals with the highest induction observed at the 5 mg/Kg/day.

Table 1 summarises the demographic characteristics and CSF biochemical variables of the AD patients at baseline, and one month into treatment. Blinded genotype analysis revealed that four patients exhibited the apoE3/3 genotype whereas the remaining seven subjects belonged to the apoE 4/3 genotype. Plasma cholesterol levels were shown to be significantly reduced after 6 months compared to baseline values ($p < 0.05$).These changes are consistent with the reported cholesterol lowering effect of Probucol in hycholesterolemic subjects.[4] Analysis of apoE levels in the cerebrospinal fluid of AD patients at baseline and month 1 revealed that Probucol was effective in increasing apoE concentration in some, but not in all AD patients.

Induction of apoE concentrations by Probucol varied from 0 to 77% ($p < 0.05$, ANOVA analysis, n=10). The response showed genotype influence: the apoE3/3 subjects exhibiting the strongest induction when compared to E4 carriers ($p = 0.09$). Tau, beta-amyloid 1-40 and lipid peroxides levels remain unchanged in response to the Probucol treatment (Table 1).

Mean clinical responses indicate a relative stabilization of the progression of the disease on the cognitive and the global improvement scales during the 6 month trial. Figure 1 illustrates the results of the correlational analysis that was performed on CSF apoE concentrations as a function of clinical response using the ADAS-Cog scale. It reveals a statistically significant association between the two variables whereby apoE induction above the 35% threshold appears to be associated with clinical improvement ($p < 0.05$, using Multiple General Linear Model analysis, SPSS Inc.). While two subjects were left out of this particular analysis because of poor compliance in the second portion of the trial, they were included in the group analyses of ADAS-Cog and DAD (Table 1).

Table 1. Demographic characteristics of the AD subjects enrolled in the Probucol clinical drug trial. Biochemical and clinical outcome measures were obtained after 1, 3, and 6 months of treatment

	Baseline	+/-	SEM	Month 3	+/-	SEM	Month 6	+/-	SEM	Difference	
Clinical Response											
ADAS-Cog	24.45	+/-	2.35	25.64	+/-	2.51	25.6	+/-	3.27	NS	
DAD	78.64	+/-	3.56	85.45	+/-	3.14	82.20	+/-	5.98	NS	
MMSE	17.64	+/-	0.96	---			16.00	+/-	1.35	NS	
Biological Markers in the Cerebrospinal fluid											
	Baseline (N=10)	+/-	SEM	Month 1 (N=10)	+/-	SEM	Ratio Month 1/Baseline Mean (%)			Difference	
Apo E	6.52	+/-	0.90	7.75	+/-	1.10	118,94%			p=0.046	*
ApoE4 Carriers	6.08	+/-	1.24	6.52	+/-	1.30	107,32%			NS	
ApoE3/2 Carriers	7.70	+/-	1.83	11.04	+/-	1.99	143,40%			p=0.09	**
Tau	658.20	+/-	112.39	666.25	+/-	114.18	101,22%			NS	
Lipid Peroxides	0.71	+/-	0.01	0.7	+/-	0.03	98,35%			NS	
Beta Amyloid 1-40	30.31	+/-	2.49	32.32	+/-	2.70	106,63%			NS	
Biological Marker in the Blood											
	Baseline (N=10)	+/-	SEM	3 Months (N=10)	+/-	SEM	6 Months (N=10)	+/-	SEM	Difference	
Cholesterol	5.06	+/-	0.37	4.64	+/-	0.33	4.34	+/-	0.33	p<0.05	***

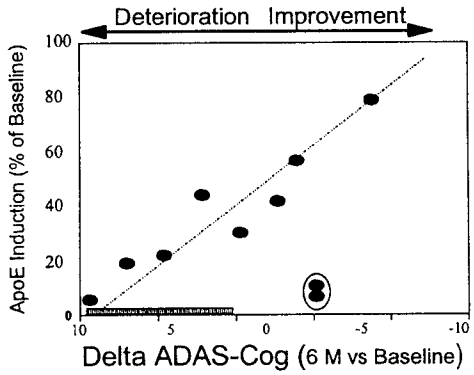

Figure 1. Effect of Probucol-mediated apoE induction in the cerebrospinal fluid of AD subjects at month one as a function of clinical response at month 6, using the ADAS-Cog scale. Shadow area highlights historical placebo range for subjects enrolled in memory enhancer drug trials. Coefficient correlation : 0.51 and p=0.031 (MGLM, SPSS Inc).

4. DISCUSSION

The crucial role played by apoE during normal brain reinnervation and the near complete absence of plasticity in the brain of apoE knockout mice, in apoE4 knockin mice and in apoE4/4 AD subjects point toward lipid delivery as one of the rate limiting steps in neuronal remodelling.[5] The correlation that exists between ADAS-Cog variation and apoE induction highlights the importance of apoE as an important surrogate biological marker to be used in the etiological treatment of Alzheimer's disease. The evidence reported here indicate the pharmacological concentrations of Probucol can promote apoE synthesis in the brain of AD patients without affecting steady state concentrations of Tau, beta amyloid 1-40 or lipid hydroperoxides in the CSF. We cannot exclude the possibility that additional Probucol activities might also be relevant, including modulation of 1) cholesteryl ester transfer protein, 2) other apolipoproteins (apoA-1, apoC-I) and 3) neurotrophin signalling.[2] The exact biological basis for the induction of apoE production in response to Probucol is currently unknown. The concomitant and timely raise of apoE mRNA prevalence and apoE protein secretion in the CNS is certainly consistent with a direct modulatory effect on gene expression *in vivo*. Moreover, circulating levels of cholesterol were found to be significantly reduced by Probucol after 6 months in AD subjects; highlighting an important beneficial side effect to the overall disease stabilization profile of Probucol. This observation is quite consistent with recent reports that the use of cholesterol lowering agents called statins reduced the prevalence of AD by 60 to 72% in a 57,104 elderly subjects cohort from the US.[6] Similar results were reported more recently in the Boston area.[7] The combination of these results with those above clearly suggest that chronic administration of Probucol could be used to activate the beta amyloid scavenging activity of apoE to indirectly reduce amyloid deposition *in vivo* in the CNS.

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