Apolipoprotein E represents a potent gene-based therapeutic target for the treatment of sporadic Alzheimer’s disease

Judes Poirier*

McGill Centre for Studies in Aging and Douglas Mental Health University Institute, Montreal, Quebec, Canada

1. Introduction

Alzheimer’s disease (AD) is associated with neuronal loss, synaptic damage, deposition of β-amyloid, and loss of cholinergic activity in susceptible brain regions. These three neuropathologic markers of AD are markedly modulated by the presence of the apolipoprotein e4 (APOE e4) allele in sporadic AD subjects. The APOE e4 allele is also a well-known risk factor for sporadic late-onset AD. Patients born with two E4 alleles were shown to exhibit (1) an earlier age of onset, (2) higher amyloid plaque counts [1–3], (3) cerebrovascular amyloid deposition [2], and (4) marked reductions in choline acetyltransferase [4–7], nicotinic, and nerve growth factor receptor [5] density compared with non-e4 allele subjects.

Recent clinical evidence clearly indicates that the APOE polymorphism significantly affects the rate of conversion from mild cognitive impairment (MCI) to probable AD [8]. It concomitantly affects the decline in cognitive performance of MCI subjects in an APOE e4 allele dose-dependent manner on cognitive performance (according to the Alzheimer’s Disease Assessment Scale, cognitive subscale [ADAS-cog]) in 2-to-4 year follow-up studies with donepezil, rivastigmine, and galantamine [8–10]. Moreover, it was shown that whereas reductions of hippocampal and entorhinal cortex volumes appear to act as predictors of conversion from MCI to AD, they do so only in APOE e4 carriers [10,11].

When one examines the effect of the APOE e4 allele on rate of conversion to AD, rate of decline, synaptic remodeling, pathologic accumulation of amyloid, and even on extent of cholinergic drug response in sporadic AD subjects, it is tempting to postulate that specific pharmacologic manipulations designed to counter the impact of APOE e4 on the physiology of the adult central nervous system (CNS) are bound to provide beneficial relief to both “genetically at risk” subjects and patients with a clinical diagnostic of possible or probable AD.

2. Proposed apoE functions in the mature brain

Several different hypotheses were suggested to explain how the e4 allele modulates the age of onset, clinical progression, and conversion from MCI to AD status. These include perturbations of (1) cholesterol/phospholipid homeostasis and synaptic integrity [12,13], (2) amyloid metabolism [14], (3) tau phosphorylation and tangle accumulation [15,16], and neuronal survival through the generation of proteolytic breakdown products [17–19].

All these working hypotheses propose interesting links between established pathologic landmarks of sporadic AD and postulated functions attributed to APOE e4 in the brain. However, none of these, except one, provides a testable biochemical explanation for the observed effect of the e4 allele on the onset and progression of AD, and the clinical response to memory-enhancing medication: the “APOE e4 lipid imbalance hypothesis” [13]. Furthermore, the proposed mechanism must take into consideration the fact that the clinical manifestations of APOE e4 are silent for more than six decades.

The lipid imbalance hypothesis was first presented by our group some 14 years ago [13]. It was based on the observation that one of the apoE core functions in the brain is to transport cholesterol and phospholipids from astrocytes to neurons. During development, and in response to brain damage or neurodegeneration in adults, apoE is actively involved in the recycling of cholesterol from dead or dying cells to neurons undergoing terminal remodeling and synaptic replacement [20]. In absence of apoE, such as in apoE knockout mice, the recycling/reinnervating system is inoperative. Similarly, in the absence of apoE’s main receptor, i.e., the low-density lipoprotein receptor, synaptic remodeling and plasticity are gravely impaired [21]. Consequently, synapses progressively lose their integrity with aging in apoE-deficient mice [22], the reinnervation process becomes markedly impaired with age [23,24],

*Corresponding author. Tel.: 450-766-2010; Fax: 450-888-4094.
E-mail address: judes.poirier@mcgill.ca

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and cognitive impairment progresses to a point of no return by age 10 months (middle-aged mice) [25,26].

Introduction of human APOE e3 or APOE e4 genes into apoE knockout mice completely prevents the cognitive deficit that characterizes apoE-deficient mice [27,28]. More interestingly, crossbreeding of APOE e4 or APOE e3 mice with APP-717 amyloid overexpresser transgenic mice almost completely abolishes the characteristic accumulation of β-amyloid deposits reported in hippocampal and cortical areas [29]. This is completely consistent with the proposed notion that apoE in the brain acts as an active scavenger of extracellular amyloid in the extracellular space [30,31].

3. Is there a biological link between APOE polymorphisms and APOE concentrations in the brain?

The answer to this question actually came from the cardiovascular field more than 25 years ago. Utermann et al. [32], using young, healthy control subjects, reported that plasma concentrations of apoE were highly dependent on the isoform expressed by the subject. Humans with an APOE e2/e2 genotype express the highest levels of apoE of all humans, whereas those with an APOE e4/e4 genotype display the lowest concentrations of all humans. Interestingly, there is an inverse correlation between risk levels and APOE e2 < e3 < e4 [33], suggesting that those with low apoE levels (APOE e4/e4) seem to be at higher risk, whereas those with the highest levels (APOE e2/e2) display the lowest level of risk [34]. Subjects born carriers of the APOE e2 allele are also predisposed to become centenarians [35,36].

We carefully repeated these analyses in both plasma and brain tissues of large cohorts of autopsy-confirmed AD cases [37] and mild-to-moderate probable/possible AD patients [38], and found identical genotype-dependent apoE concentration gradients in sporadic AD subject (Fig. 1),
where the highest CNS apoE levels were found in APOE e2 carriers (very rare in AD), and the lowest levels in APOE e4 cases (the majority of AD patients).

4. **APOE: A logical therapeutic target**

At the moment, two opposite views prevail in the literature with regard to the possible mode of action of APOE e4 in the CNS. One view holds that APOE e4 is bad per se, and that it is toxic to neurons but not to other cells in the body, and that somehow this toxicity is latent for several decades in e4 carriers. The other point of view, which is less pessimistic, is articulated around the fact the APOE e4 allele is the ancestral form of the gene. Actually, it is the one found in all other mammals examined so far, including several primate species. Contrary to prevailing views, it is the APOE e3 and APOE e2 variants that humans have acquired in the past few hundred thousand years [39]. In this model, APOE e4 is not detrimental per se. Instead, it performs its duties in humans as well as in all other APOE e4 mammals. However, in humans, low levels of apoE characterize APOE e4 allele carriers and the poor ability of the e4 genetic variant to respond to physiologic inducers of expression, which is actually problematic in aging subjects. It is postulated that the presence of the e4 allele progressively compromises brain lipid homeostatic processes during normal aging, and more so in brains suffering from damage or degenerative conditions [13].

In this model, we postulate that the restoration of apoE concentrations in the brains of APOE e4 carriers to levels found normally in APOE e3 subjects (or APOE e2 subjects) would either delay disease onset or slow down the rate of progression. We previously demonstrated that the ability of APOE e4 to bind to its receptors, and to promote lipoprotein uptake in brain cells, is similar to that of APOE e3 in vitro. We believe that levels are at stake here, and because apoE concentrations are vital to the proper maintenance of synaptic integrity and remodeling, the first casualty of a lifelong deficit is the synaptic network of the aging brain, particularly in neurons with long, projecting axons that use massive amounts of lipids such as cholesterol and phospholipids to maintain their structural integrity during normal aging.

In an attempt to address this issue, we developed a low-throughput screening assay using primary type 1 astrocytes from rodent cortical areas, and tested a large numbers of drugs, proteins, and hormones capable of enhancing apoE synthesis or secretion in vitro. The most interesting candidates were then tested in vivo for efficacy and safety. Finally, one compound among many others that met our biochemical and physiological criteria was tested in a pilot study in mild-to-moderate AD subjects. Of several hundred compounds tested, we identified three significant up-regulators of apoE synthesis and secretion, and two repressor compounds. We also examined several members of the Statin family (cholesterol synthesis inhibitors), but
none of these modified apoE synthesis and secretion at physiologically relevant concentrations.

Estrogen, indomethacin, and probucol were found to significantly enhance apoE synthesis and secretion in vitro and in vivo in the mature rodent CNS (mice and rats). Estrogen not only enhanced apoE synthesis and secretion in vivo, but also enhanced synaptogenesis and compensatory terminal remodeling in response to deafferentation in the mature rodent hippocampus [40]. Indomethacin, a cyclooxygenase inhibitor used a few years ago to slow down the progression of AD in a placebo-controlled, double-blind, clinical trial in mild-to-moderate AD [41], was found to act as a very potent apoE inducer in subnanomolar concentrations [42]. A subsequent systematic analysis of the structure-activity relationship of several indomethacin derivatives revealed that the portion of the molecule responsible for apoE induction activity is different from the cyclooxygenase inhibitory moiety, consistent with a non-immunosuppressing mode of action, and presumably involving the peroxisome proliferating activating receptor-γ (PPAR-γ) pathway [42].

This observation is consistent with a recent clinical study by Risner et al. at GlaxoSmithKline, who reported significant clinical improvement of cognitive deficit in a large, double-blind, placebo-controlled, 6-month clinical trial in mild-to-moderate AD using PPAR-γ agonist rosiglitazone [43]. Agonists belonging to the PPAR-γ family of drugs are well-known inducers of apoE synthesis and secretion in peripheral cells, and are potent anti-inflammatory agents [44].

The most interesting compound identified during our apoE induction screening assays was undoubtedly probucol, an old cholesterol-lowering drug used to treat familial hypercholesterolemia. It is still in use in Japan. The administration of probucol (1% w/w) in rat and mouse diets, at concentrations designed to mimic the dose used in humans, caused a significant induction of apoE synthesis and secretion after 30 days in cortical and hippocampal areas (Fig. 2). Synaptic density, as measured by hippocampal GAP-43 and synaptophysin immunohistochemistry, increased during treatment (Fig. 2) in 24-month-old rats. Similar results were found in the brains of C57/Bl mice exposed to probucol (intraperitoneal) for 2 weeks (not shown).

After authorization from the Health Protection Branch of Health Canada, we set up a small pilot clinical trial to test

Fig 3. Effect of probucol administration on cerebrospinal fluid levels of total tau (top) and total β-amyloid (bottom) in probucol-treated Alzheimer’s disease subjects. Graphs represent ratio values of 1 month of treatment over baseline concentrations for each subject enrolled in the clinical trial. Adapted from Poirier and Panisset [45] and Poirier [50].
probucol in a cohort of 12 mild-to-moderate AD subjects not using acetylcholinesterase inhibitors or Memantine. This proof-of-concept clinical study examined the effects of a standard dose of probucol (500 mg/bid) used to treat hypercholesterolemia before the introduction of statins almost a decade ago. Probucol was administered in mild-to-moderate AD subjects for period of 6 months. Clinical assessments revealed a concomitant stabilization of symptoms on the ADAS-cog and on the Disability Assessment of Dementia (DAD) scale over the course of the trial [45]. The clinical benefits according to the ADAS-cog correlate very well with the increase of apoE levels in the cerebrospinal fluid (CSF) of these patients ($P < 0.05$). More interestingly, there was a significant inverse relationship between the increase in apoE levels and the reduction of total $\beta$-amyloid levels in the CSF of probucol-treated AD subjects [46] (Fig. 3).

Fig. 4A illustrates the cumulative effect of probucol administration on apoE level measured in the CSF at 1 month and at baseline. If one assumes that the overall probucol consumption was constant over the course of the trial, we can see that the impact of probucol on clinical parameters at 6 months (Fig. 4B) was more pronounced after 100 g (or 100 equivalent days of treatment), whereas the effect of probucol on apoE concentration in the CSF was...
more pronounced after 150 g (or 150 equivalent days of treatment; Fig. 4A).

These results are suggestive at best. Only a prospective, double-blind, placebo-controlled clinical trial of AD subjects with and without APOE e4 over a long period of time will allow us to confirm the beneficial effect of probucol in sporadic AD. It is interesting to note that the effect of probucol was significantly more pronounced in APOE e3 cases than in APOE e4 allele carriers [45], i.e., a pharmacogenomic response very similar to that described for rosiglitazone [43] and estrogen [47], two very potent APOE-inducer drugs, on cognitive performance in non-e4 subjects.

The recent discovery that nearly 80% of all APOE e4 MCI subjects convert into probable/possible AD cases within 3 years allows us to test the beneficial effects of apoE inducers such as probucol, estrogen, and rosiglitazone in a population heterozygous for the e4 allele, namely the APOE e4/e3 subgroup, which is characterized by (1) low circulating levels of apoE, (2) a high risk of conversion to AD, and (3) marked hippocampal and entorhinal cortical shrinkage in a relatively short-term perspective. Such a pharmacogenomic trial would not only allow us to test the effect of apoE-inducers such as probucol on the progression and conversion of the disease, but also to examine their potential use in the prevention of sporadic AD in APOE e4 carriers.

5. Conclusions

We have reviewed the apparent role of APOE e4 in the development of dementia and AD pathology. Although age remains a key determinant that modulates the onset and expression of AD pathology, genetic risk factors such as APOE appear to play a central role in the pathophysiology of this disease, for years, if not decades, before clinical diagnosis. The combined use of genetic profiling and gene-targeting will allow us to better identify the biochemical mechanisms regulating the loss of synaptic integrity and the accumulation of amyloid deposits in the aging brain. The recent discovery that estrogen and rosiglitazone, multifunctional agents with common apoE-inducing properties, perform best in APOE e3/e3 AD patients is consistent with early data from the probucol AD trial that also showed preferential benefits for APOE e3/e3 AD cases. The surprising convergence of these biochemical, pharmacogenomic, and clinical observations raises exciting new possibilities and certainly interesting new therapeutic avenues for the treatment and prevention of a genetically defined and sizeable subset of AD subjects.

References


