Therapeutic approach targeting apolipoprotein E binding region and low-density lipoprotein receptor for Alzheimer’s disease

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ABSTRACT

Approximately 13% of the population over the age of 65 years is estimated to have AD. The total number of cases is expected to increase over the coming decades. The apolipoprotein E (ApoE) genotype is the greatest genetic determinant for Alzheimer’s disease (AD) development. The ApoE4 allele increases the risk of AD by 4 to 14 fold while the ApoE2 allele has an opposing effect; decreasing risk. Indeed many studies have demonstrated that carriers of the ApoE2 allele are associated with greater likelihood of survival to advanced age, superior verbal learning ability in advanced age, and reduced accumulation of amyloid pathology in the aged brain. In addition, it is known that ApoE proteins have different affinities for the low-density lipoprotein receptor (LDLR), with ApoE2 having the weakest binding to the LDL receptor at < 2% relative to ApoE3 and E4. Because ApoE2 has shown protective effects in regard to AD, a novel approach for ApoE4 carriers may be to create a peptide antagonist that blocks the ApoE interactions with LDLR at its 135-150 N-terminal binding domain. This peptide may create a more ApoE2-like structure by decreasing the affinity of ApoE4 for LDLR thereby reducing AD onset, memory impairment, and amyloid plaque formation. In this review, we will discuss the different detrimental effects that ApoE4 can cause. Most importantly, we will review how ApoE4 binding to LDLR promotes AD pathogenesis and how blocking ApoE4 binding may be a promising novel therapeutic approach for AD.

Keywords: Alzheimer’s disease, low-density lipoprotein receptor, apolipoprotein E, amyloid precursor protein, late onset Alzheimer’s disease

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INTRODUCTION TO APOE

As life expectancies increase, more elderly patients are diagnosed with Alzheimer’s disease (AD). AD brain exhibits close to 50% neuron loss in the cortex. Genome-wide association studies (GWAS) have identified the apolipoprotein E (ApoE) gene as a risk factor for AD\(^1\)\(^2\). It has been found that there is a strong correlation between ApoE4 carriers and higher levels of amyloid pathology. However, individuals who do not carry the ApoE4 allele seem to demonstrate fewer AD disease processes or other neurodegenerative disorders\(^3\)\(^-\)\(^6\).

The human ApoE gene is encoded on chromosome 19. ApoE is a 34-kDa protein consisting of 299 amino acids and is constitutively expressed in astrocytes, microglia, vascular smooth muscle cells, and choroid plexus while neurons typically generate ApoE under stress conditions. Through mRNA studies it has been demonstrated that the liver is the major producer of ApoE, followed next by the brain. The receptor-binding domain of ApoE is located within amino acids 136-150 of the N-terminal region. There are three different human isoforms of ApoE (ApoE2, ApoE3, and ApoE4) which differ by only 2 amino acids at sites 112 and 158. ApoE2 has cysteines located at both sites, ApoE3 has a cysteine at site 112 and arginine at site 158 while ApoE4 has arginines at both sites\(^7\)\(^8\). The heterogeneous nature of the three isoforms is secondary to genetic polymorphisms\(^9\). It has been shown that there is a linear reduction in brain hippocampal volumes by magnetic resonance imaging (MRI) scans according to ApoE genotype in the following hierarchy: ApoE4 < ApoE3 < ApoE2. In AD patients, ApoE4 carriers had significantly smaller hippocampal volume compared to ApoE2 carriers. This study used several well-characterized cohorts to analyze the neuroanatomic effect of ApoE on the left and right hippocampal volumes\(^10\). In addition, research has shown that the E4 allele is also a risk factor for atherosclerosis, human immunodeficiency virus (HIV) disease progression, cerebral amyloid angiography (CAA), tauopathies, dementia with Lewy bodies, and Parkinson’s disease\(^11\).

APOE4 MECHANISM

ApoE4 increases the risk of developing AD by 4-fold with one allele and 14-fold with two alleles\(^12\). The approximate allele frequencies of E2, E3, and E4 in the human population are 7%, 78%, and 14%, respectively\(^6\)\(^13\). Moreover, it has been shown through histological analyses of AD brains that ApoE is co-deposited with amyloid-beta (A\(\beta\)) in amyloid plaques\(^14\). It has also been revealed that A\(\beta\) clearance is faster in ApoE3 transgenic mice versus ApoE4 transgenic mice\(^15\). This is likely because ApoE4 has an altered structure compared with ApoE2 or ApoE3, which alters its function. Therefore, understanding the structural properties of ApoE and its isoforms is vital to creating a prophylactic or therapeutic treatment. Research has shown that competition assays with ApoE4, ApoE3, and Tau revealed that ApoE4 inhibits Tau degradation. In addition, a single nucleotide polymorphism rs429358 defines ApoE4 and is located within exon 4 of apolipoprotein E. In regard to ApoE4, the arginine at position 112 directly influences arginine-61, which allows for domain interaction with glutamine-255. In addition, this bulky charged arginine residue destabilizes the N-terminal helix bundle domain, inducing helix shortening between amino acids 12 and 20 of the N-terminal domain and residues 204 and 210 of the C-terminal domain which reduces ApoE4 ability to form tetramers. This results in ApoE4 binding preference for very low-density lipoprotein (VLDL)\(^16\)\(^-\)\(^20\).

APOE AND THE LOW-DENSITY LIPOPROTEIN RECEPTOR INTERACTION

LDLR is one member of a family of seven core LDL receptor-related proteins (LRPs), which also includes LDLR-related protein 1 (LRP1), the VLDL receptor (VLDLR), megalin (LRP2), apolipoprotein E receptor 2 (ApoER2), and LRP4. All LDL receptor family members share structural properties that allow interaction with ApoE\(^21\). In addition, LDL receptor family members contain a transmembrane domain which can be endocytosed, proteolytically processed, and interact with cell proteins, including direct interaction with (amyloid precursor protein) APP\(^22\). LDLR, VLDLR, LRP, and ApoER2 are present in a number of brain cells including astrocytes, microglia, neurons, and oligodendrocytes\(^23\). It has also been reported that overexpres-
sion of LDLR decreases ApoE levels in the brain, while LDLR deficient mice have increased ApoE brain accumulation[24,25]. Further, LDLR overexpression elevates uptake of Aβ in astrocytes. Conversely, deletion of LDLR has an opposing effect[26]. Upon culturing brain sections with Aβ plaques with murine astrocytes, Aβ was taken up and degraded via LDL receptor or LDL receptor related protein[27]. ApoE contains 299 residues and was identified as a main component of lipoproteins in plasma. It has been established that lysine and arginine residues situated between ApoE residues 136 and 150 interact directly with acidic residues in the ligand binding domain of LDLR. In addition, full receptor binding activity requires arginine at position 172 located at the hinge region that connects the N- and C-terminal domains. ApoE3 and ApoE4 bind to LDL receptors with high affinity, but the binding of ApoE2 is 50- to 100-times weaker[28]. These data suggest that ApoE4 confers the highest risk for AD pathology due to its increased affinity for LDLR.

Recent research has shown that ApoE binding to ApoE receptors increases transcription of Aβ through activation of the mitogen activated protein (MAP) kinase signaling pathway involving dual leucine-zipper kinase (DLK). In fact, ApoE binding to cell-surface ApoE receptors activates DLK. The levels of Aβ potency production increase according to the different human ApoE isoforms (ApoE4 > ApoE3 > ApoE2). Specifically, when ApoE binds to ApoE receptor, DLK is activated. DLK will then activates dual specificity mitogen-activated protein kinase 7 (M KK7) and extracellular signal-regulated protein kinase (ERK) 1/2 MAP kinases. Further more, activated ERK1/2 induces cFos phosphorylation, that will eventually stimulate the transcription factor activation protein (AP)-1. Transcription factor AP-1 will enhance transcription of APP and thereby increase Aβ levels[29]. Therefore, a peptide or antibody blocking the interaction between LDLR and the ApoE binding site may potentially decrease the MAP kinase cascade and APP transcription, ultimately leading to a decrease in Aβ production. Previous research demonstrated that the monoclonal antibody 1D7 is specific for human ApoE and blocks binding of lipid-associated ApoE to LDLR[30]. 2E8 monoclonal antibody also binds to ApoE and prevents ApoE-mediated binding of lipoproteins to the LDLR[31].

**APOE2-LIKE PROPERTIES AND BENEFITS**

Although ApoE2 known to cause type III hyperlipoproteinemia, the E2 allele is known for being protective against the development of late onset Alzheimer’s disease (LOAD) compared to the common E3 and E4 allele as exemplified by a delayed age of onset and a greater likelihood of survival to advanced age. A cross-sectional multimodal neuroimaging approach has shown ApoE2 to be protective in the aged brain. In addition, the ApoE2 allele appears to have a relatively selective effect on reduced accumulation of amyloid pathology in the aged brain[12-14]. It has been reported that ApoE2 can promote type III hypercholesterolemia, leading to increased cardiovascular disease. However, studies demonstrate that ApoE4 knock-in mice have lower than normal brain cholesterol concentrations even though peripheral cholesterol levels are increased. This finding suggests that brain ApoE metabolism is distinct from that in the plasma. Moreover, the blood-brain barrier (BBB) effectively prevents the exchange of brain tissue and plasma lipoproteins. Thus, peripheral cholesterol cannot cross the BBB and enter the brain. Brain cholesterol is mainly synthesized in situ and provided by *de novo* synthesis, primarily by astrocytes and oligodendrocytes[11,32-35].

ApoE2 is associated with slower cognitive decline, milder Aβ pathology, and less neurodegeneration compared to ApoE3 and ApoE4. Older individuals who are ApoE2 carriers display superior verbal learning abilities, and faster processing of information. Possession of at least one copy of the ApoE2 allele has demonstrated a slower decline in episodic memory[34,35]. All isoforms of ApoE can modulate Aβ clearance. However, aging APP transgenic mice expressing human ApoE2 also have the slowest rate of production of Aβ oligomers with neuritic plaque formation compared to ApoE3 and ApoE4 mice[36].

Rats expressing human ApoE2 have been shown to be protected from apoptotic death of cortical neurons induced by Aβ peptides[37]. ApoE2 mice are also more effective in clearing Aβ from the bloodstream and pro-
moting degradation of Aβ. In addition, ApoE2 carriers have increased dendritic outgrowth, which enhances the formation of new synapses and can protect against AD synaptic deterioration[34,39]. Further, ApoE2 protected cultured cells most effectively, compared to the other ApoE isoforms, from oxidative stress-induced death in vitro[40]. The cysteine to arginine substitution at position 158 in ApoE2 makes ApoE2 more stable to thermal and chemical denaturation, compared to ApoE3 and ApoE4. Moreover, the cysteine residue at position 112 creates a lesser chance to exhibit domain interactions relative to ApoE4[34,41,42]. It has been suggested that the development of drugs that can prevent the domain interaction of ApoE4 and convert ApoE4 to a more ApoE3/ApoE2-like structure may be beneficial for individuals with neurodegenerative disorders. In addition, a peptide blocking the 135-150 N-terminal region may create a more ApoE2-like structure, as ApoE2 has decreased affinity for the LDLR. Given that ApoE2 carriers have a lower risk and delayed age of onset of AD compared to E3 and E4 carriers[11,34,43], it would stand to reason that creating a more ApoE2 structure can be beneficial for treating AD rather than using ApoE E3 or ApoE4 structures.

CONCLUSION

Currently approximately 5.1 million Americans are affected with AD and the number is expected to triple by 2050. Further there are no truly effective disease-modifying therapies for AD. ApoE4 is known to play a major role not only in AD, but also atherosclerosis, CAA, tauopathies, dementia with Lewy bodies, and stroke. Approximately the allele frequencies of E2, E3, and E4 in the human population are 7%, 78%, and 14%, respectively. ApoE genotypes have different affinities for LDLR, with ApoE2 having the weakest binding to LDLR at ApoE3 > ApoE2[8,11,13,16,43-45]. We suggest that a peptide targeting the ApoE LDLR binding domain may work as a competitive antagonist for patients who are ApoE4 carriers, in effect creating a more ApoE2-like structure[Figure 1].

Creating a more ApoE2-like structure may be associated with greater likelihood of survival to advanced
age, superior verbal learning abilities, improved recall memory, faster processing of information, better test performance, and reduced accumulation of amyloid pathology in the aged brain. Furthermore, a second innovative approach would be to create a more advanced antibody targeting specifically the 133-152 N-terminal binding region of ApoE to prevent interaction between LDLR and ApoE. In sum, modulation of ApoE structure to create and/or enhance ApoE2-like activity may shed light on a novel approach for AD treatment and prevention.

DECLARATIONS

Authors’ contributions
Reviewed the literature and wrote this article: Leon M
Edited and added further information to this article: Sawmiller D, Giunta B
Contributed to the initial idea of the review: Tan J
Read and approved this article: all authors

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