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REVIEW

Apolipoprotein E, amyloid-β clearance and therapeutic opportunities in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterised by extracellular amyloid-β (Aβ) and intraneuronal tau protein brain pathologies. The most significant risk factor for non-familial AD is the presence of the E4 isoform of the cholesterol transporter apolipoprotein E (apoE). Despite extensive basic research, the exact role of apoE in disease aetiology remains unclear. Correspondingly, therapeutic targeting of apoE in AD is at an early preclinical stage. In this review, I discuss the key interactions of apoE and Aβ pathology, the current progress of preclinical animal models and the caveats of existing therapeutic approaches targeting apoE. Finally, novel Alzheimer's genetics and Aβ-independent disease mechanisms are highlighted.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in aged populations, being characterized by cerebrovascular and neuronal dysfunctions that induce a progressive decline in cognitive functions [1]. The occurrence of AD in individuals aged over 65 years is defined as late-onset AD (LOAD) - representing the majority of AD sufferers. Patients with early-onset AD (EOAD) represent approximately 1% of the overall population [2].

Symptomatic AD is diagnosed clinically using a battery of cognitive tests, with significant efforts ongoing to move diagnosis to earlier disease stages using the additional tools of genetic testing, blood and cerebrospinal fluid biomarkers and neuroimaging [3]. Previous to these advances, however, AD could only be definitively diagnosed as the cause of dementia by post-mortem detection of two major neuropathologies. These comprise senile

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plaques of aggregated Aβ peptide, and neurofibrillary tangles of hyperphosphorylated, aggregated tau protein.

Amyloid-β

Aβ peptides are produced through sequential proteolysis of the amyloid precursor protein (APP) by β-secretase/BACE and the γ-secretase complex (partly comprising the presenilin PS1 or PS2). Aβ peptides vary in length from 39 to 43 amino acids with the predominant species being Aβ40 and Aβ42 [4]. Disease-modifying AD drug discovery research has focused on strategies targeting production or clearance of the Aβ peptide. This 'amyloid hypothesis' has been driven by the fact that familial EOAD with autosomal dominant inheritance is caused by mutations in the *APP*, *PS1* or *PS2* genes. In simple terms, the net effect of these mutations is to increase either bulk Aβ levels or the ratio of Aβ42:Aβ40 production [5]. An increase in brain Aβ42 levels, whether absolute or ratiometric, is hence critical to the aetiology of familial EOAD.

In agreement with the amyloid hypothesis, studies in transgenic mouse models of AD imply a cascade of events in which abnormal forms of tau act as downstream mediators of Aβ toxicity [6,7]. Contrary to this proposed cascade, however, whilst neuronal loss and neurofibrillary tangle counts strongly predict cognitive status in LOAD patients, total Aβ plaque load correlates weakly with cognitive impairment [8]. The prevalent explanation for this disparity is that it is diffusible Aβ oligomers, rather than Aβ plaques, that represent the actual toxic species. The E693Δ APP mutation, for example, causes Alzheimer's-type dementia through the toxicity of non-fibrillar, intracellular Aβ oligomers [9]. Conversely, the 'Arctic' APP mutation (E693G) induces formation of large Aβ oligomers known as protofibrils [10]. Experimental disagreement over the physicochemical nature of toxic oligomers in LOAD has hampered delineation of their exact role in disease [11].

Apolipoprotein E

Apolipoprotein E (apoE) is the primary transporter of cholesterol in the central nervous system (CNS), being synthesised within the blood brain barrier (BBB)

predominantly by astrocytes [12]. Three apoE poly-morphic alleles (*APOE2*, *APOE3*, and *APOE4*) encode three protein isoforms (apoE2, apoE3 and apoE4) that differ by cysteine/arginine polymorphisms at position 112 or 158. The *APOE4* allele, found in 15% of the popu-lation, remains the most significant genetic risk factor for LOAD [13].

In support of the amyloid hypothesis, *APOE4* carrier status is associated with greater Aβ plaque load in both AD patients and cognitively normal individuals [14,15]. The *APOE4* allele also correlates with increased cerebro-vascular Aβ deposition [16] and, correspondingly, is a risk factor for cerebral amyloid angiopathy [17]. As a consequence, research into the mechanistic connection between apoE4 and LOAD has focused on delineating the interaction of apoE with Aβ pathology (Figure 1). Experimental data now support a clear and necessary role for apoE in Aβ toxicity.

Interactions of ApoE with Aβ pathology

In vitro studies have demonstrated that apoE4 more than apoE3 interacts directly with Aβ [18], enhancing Aβ fibrillisation [19]. Interpretation of such data is compli-cated by the difficulties of replicating *in vivo* Aβ conformation and apoE lipidation status. However, early Aβ amyloidosis mouse model data also support a clear role for apoE in Aβ pathology [20]. As a consequence of these findings, apoE/Aβ interaction inhibitors are being developed as AD therapeutics. Small Aβ-mimetic peptides intially demonstrated reductions in apoE-stimulated formation of neurotoxic Aβ aggregates *in vitro* [21], with these data being subsequently confirmed *in vivo* using a mouse model of Aβ brain amyloidosis [22].

ApoE proteins comprise an amino-terminal receptor-interacting domain and carboxy-terminal lipid-binding domain. Fluorescence lifetime imaging-fluorescence resonance energy transfer (FLIM-FRET) studies on human post-mortem tissue sections indicate that Aβ is prefer-entially associated with the carboxyl terminus of apoE4 compared to that of apoE3, and that apoE4 undergoes greater amino-terminal degradation, prolonging Aβ inter-action [23]. This prolonged interaction may enhance formation and stabilisation of toxic Aβ oligomers [24]. Analyses of AD brain samples have demonstrated a higher burden of oligomeric Aβ in *APOE4* carriers with increased amyloid plaque-associated synaptic loss. ApoE4 colocalises with oligomeric Aβ at the synapse, indicating a key role as a co-factor in Aβ toxicity [25].

The greater susceptibility of apoE4 to proteolytic cleavage, and the subsequent prolongation of Aβ inter-actions, is thought to be a consequence of differential domain interaction. The C112R polymorphism in apoE4 results in a salt bridge between R61 and E255, which is lacking in apoE3 [26]. This brings the amino- and carboxy-terminal domains into closer proximity and exposes the hinge region of apoE4 to proteolysis [23]. Consequently, the development of small-molecule 'struc-ture correctors' that shift apoE4 to an apoE3-like confor-mation has also been proposed as a therapeutic strategy for AD [27].
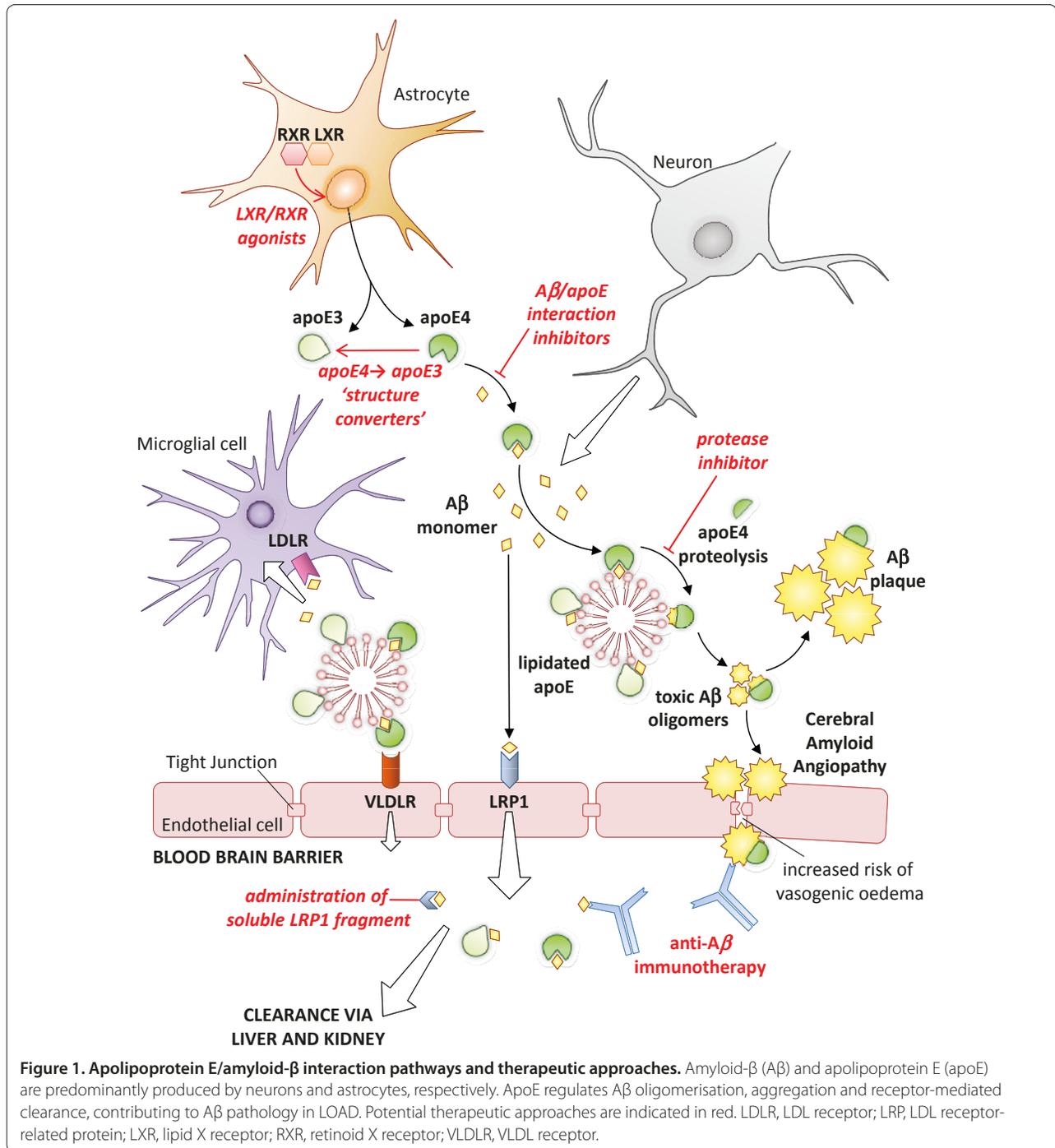
The main challenge for small molecule approaches aiming to disrupt apoE intradomain or apoE/Aβ protein-protein interactions is to achieve a compound with sufficient potency, specificity and BBB permeability to be suitable for clinical trials.

ApoE mouse models of Aβ amyloidosis

Multiple mouse models of Aβ brain amyloidosis have been generated, predominantly comprising familial, EOAD APP and PS1/2 mutations either alone or in com-bination [28]. To varying degrees, these mice recapitulate brain parenchymal and cerebrovascular Aβ deposition with cognitive behavioural disorder; however, neuronal loss is relatively lacking in most models. When con-sidering the impact of apoE on Aβ pathology in these mice it is important to consider that endogenous murine apoE is non-polymorphic and does not display domain interaction [29]. Consequently, mouse apoE behaves most similarly to human apoE3. In order to determine the effects of human apoE isoforms, Aβ amyloidosis transgenics have now been combined with a variety of human apoE mouse models. These crosses display delayed onset of Aβ pathology relative to their murine equivalents, emphasising the importance of interspecies differences [30].

Mice expressing mutant V717F APP in conjunction with human apoE isoform knock-ins (PDAPP/TRE mice) show isoform-dependent Aβ deposition, with apoE4 showing the strongest effect followed by apoE3 and then apoE2 [31].

Gene dosage is critically important, with haplo-insufficiency of both human apoE3 and apoE4 knock-in isoforms causing marked reductions in Aβ deposition in APP/PS1 mutant mice [32,33]. This is a key point, as there is an ongoing debate regarding the potential thera-peutic benefits of raising versus lowering apoE expression levels. Whilst the transgenic data indicate that reducing apoE levels would be more beneficial, small-molecule upregulation of apoE levels, particularly through agonism of the lipid X receptor (LXR) [34] or retinoid X receptor (RXR) [35], has been reported as a promising therapeutic approach. *In vivo* studies of such agonists, whilst successfully demonstrating reductions in Aβ pathology, were carried out against a background of endogenous murine apoE. It remains a possibility, therefore, that increasing expression of human apoE4 may actually be deleterious to disease. It should also be noted that LXR/RXR agonism has side effects, such as hypertriglyceridaemia,

Figure 1. Apolipoprotein E/amyloid-β interaction pathways and therapeutic approaches. Amyloid-β (Aβ) and apolipoprotein E (apoE) are predominantly produced by neurons and astrocytes, respectively. ApoE regulates Aβ oligomerisation, aggregation and receptor-mediated clearance, contributing to Aβ pathology in LOAD. Potential therapeutic approaches are indicated in red. LDLR, LDL receptor; LRP, LDL receptor-related protein; LXR, lipid X receptor; RXR, retinoid X receptor; VLDLR, VLDL receptor.

and the relatively hydrophobic nature of ligands makes complicating interactions with the γ-secretase multi-span membrane complex a possibility [36].

ApoE and Aβ production

There is limited evidence for modulation of Aβ production by apoE with *in vitro* studies using cultured cells co-overexpressing apoE and APP - a relatively unphysiological paradigm [37]. ApoE4-induced increases in Aβ

production could be mediated by a novel, apoE-interacting protein, TMC22, proposed to facilitate an interaction between APP and the γ-secretase complex [38].

ApoE and Aβ aggregation

Neprilysin is the major protease mediating brain Aβ degradation [39]. *In vivo* inhibition of neprilysin by thiorphan infusion induces apoE isoform-dependent

aggregation of Aβ, with apoE4 causing the greatest increase in aggregation [40]. It is possible that apoE acts to stabilise oligomeric Aβ, causing enhanced toxicity and seeding deposition of larger aggregates [24].

ApoE and Aβ clearance

Aβ is cleared from the brain by proteolytic degradation [41], bulk flow along the perivascular interstitial fluid drainage pathway [42], or by receptor-mediated clearance across the BBB [43]. In addition, the 'peripheral sink' hypothesis postulates that clearance of Aβ from the brain is accelerated by removal of Aβ from the plasma via the liver and kidneys [44]. *APOE4* carriers may display clearance deficits in both compartments as Aβ removal from both the CNS and the plasma is reduced in human apoE4 knock-in mice [31,45].

ApoE isoform status may influence CNS Aβ degradation through indirect mechanisms such as regulation of cellular cholesterol - enhancing endocytosis and lysosomal degradation of Aβ [46]. The major impact of apoE is, however, likely to be through interaction of Aβ with cell-surface apoE receptors, including LDL receptor-related protein 1 (LRP1), the LDL receptor (LDLR) and the VLDL receptor (VLDLR) [47]. Receptor binding of Aβ, alone or in complex with apoE, either delivers Aβ to the lysosome or leads to transcytosis into the plasma via the BBB. LRP1 is perhaps the best characterised transporter acting in the latter instance [48]. ApoE isoforms (apoE4 > apoE3 > apoE2) may disrupt rapid, LRP1-mediated clearance of unbound Aβ by diverting it to the VLDLR, which has a slower rate of endocytosis [49].

From a therapeutic perspective, peripheral administration of soluble fragments of LRP1 has been shown to reduce brain Aβ load in K670N/M671L APP mice through plasma Aβ binding - theoretically exploiting the peripheral sink hypothesis [50]. However, the primary investigation of this type of approach has been through enhancement of peripheral Aβ clearance through anti-Aβ immunisation strategies. These remain, despite early setbacks, one of the most promising current therapeutic avenues. Passive immunisation with the humanised anti-Aβ antibody bapinuezumab demonstrated lower efficacy in *APOE4* carriers with a corresponding increase in vasogenic oedema, suggestive of transient increases in vascular permeability [51,52]. If phase III trials are positive, determination of *APOE* status is likely to become an important aspect of treatment.

In addition to LRP1, LDLR has also been implicated in Aβ removal from the CNS. LDLR over-expression decreased Aβ deposition and enhanced clearance in the K670N/M671L APP, ΔE9 PS1 amyloidosis mouse model [53]. LDLR knockout data are inconsistent, however, as whilst two studies reported increased Aβ load [54,55] a further analysis failed to show any effect [56]. Although

LDLR-upregulating compounds have been reported [57], clinical usage of such drugs would be challenging due to specificity and toxicity concerns.

Aβ-independent disease mechanisms

Collaborative large-scale genome-wide association studies have identified, in addition to apoE, novel LOAD risk genes. These include *CLU* (encoding apolipoprotein J), *PICALM*, *CR1* and *BIN1* [58]. Conversely, variants of APP and PS1/2, which increase Aβ42 production in familial EOAD, were not hits in these studies. The genetic drivers of LOAD and EOAD are hence likely to be different. Whilst the novel LOAD risk genes may function in either Aβ clearance [43,59] or toxicity [60], there remains a possibility that key implicated pathways, such as lipid homeostasis and innate immunity, play Aβ-independent roles in the aetiology of LOAD. ApoE is linked to autoimmune inflammation, diabetes and coronary heart disease - environmental risk factors for LOAD magnified by the *APOE4* genotype [61]. The clinical failures of non-steroidal anti-inflammatories [62], a per-oxisome proliferator-activated receptor (PPAR)γ agonist [63] and HMG-CoA reductase inhibitors [64] suggest, however, that targeting mid-life risk factors for LOAD in late stage disease is unlikely to be therapeutically successful. Such treatments, including apoE-based therapeutics, may need to be given earlier in the disease process. This places additional importance on early diagnosis of AD and/or preventative treatment in individuals at high risk of developing LOAD.

ApoE, and related cell signalling, is also purported to modulate synaptic plasticity, tau phosphorylation, and neuroinflammation [47]. The extent to which apoE drives the aetiology of LOAD through these mechanisms is unclear; however, apoE mimetic peptides designed to mediate putative, beneficial effects of apoE demonstrated both behavioural and pathological benefits in mutant APP mice [65]. The main challenge with such an approach will be to achieve a candidate molecule with appropriate physicochemical properties for clinical use.

Conclusions

Understanding of the interplay between *APOE* genotype and Aβ pathology has progressed significantly in recent years, particularly with respect to human apoE knock-in animal models of Aβ amyloidosis. These demonstrate an isoform-specific role for apoE4 in retarding Aβ clearance from the CNS. By virtue of the nature of the target, however, apoE therapeutics are still at an early preclinical stage, with appreciable chemistry challenges facing small-molecule approaches. The most immediate impact of apoE on AD therapeutics will likely be the profiling of patients for *APOE4* status to help determine dosing of anti-Aβ immunotherapy treatments. ApoE has multiple

systemic functions, some of which relate to novel LOAD risk genes, which may also affect the aetiology of AD independently of Aβ. The understanding, and modelling, of these functions remain goals for future research.

Abbreviations

Aβ, amyloid-β peptide; AD, Alzheimer's disease; apoE, apolipoprotein E; APP, amyloid precursor protein; BBB, blood brain barrier; CNS, central nervous system; EOAD, early onset Alzheimer's disease; LDL, low-density lipoprotein; LDLR, LDL receptor; LOAD, late onset Alzheimer's disease; LRP, LDL receptor-related protein; PS, presenilin; VLDLR, VLDL receptor.

Competing interests

Adam Kline was in the past 5 years an employee of Eisai Limited and received a fixed salary. Adam Kline was not an Eisai employee at the time of publication.

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