Links between ApoE, brain cholesterol metabolism, tau and amyloid β-peptide in patients with cognitive impairment

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Abstract

Brain neurons remove the excess of cholesterol via conversion into the more polar 24OHC [(24S)-hydroxysterol]. 24OHC acts as a signalling molecule inducing ApoE (apolipoprotein E)-mediated cholesterol efflux from astrocytes, by a direct effect on ApoE transcription, protein synthesis and secretion. In CSF (cerebrospinal fluid) collected from patients with cognitive impairment (Alzheimer’s disease and patients with mild cognitive impairment) the levels of ApoE, tau, p-tau (hyperphosphorylated tau) were significantly increased, together with 24OHC, compared with controls. We also found that the levels of tau and p-tau were significantly correlated with ApoE and 24OHC in the same samples. Such a correlation was not found in control patients. Increased levels of cholesterol in membranes and impairment in brain cholesterol metabolism were found to be involved both in APP (amyloid precursor protein) processing and amyloid β-peptide deposition and, recently, in tau pathology. The CSF tau levels are considered to be related to the neurodegenerative process in Alzheimer’s disease. During neurodegeneration, the cholesterol accumulated in neurons is converted into 24OHC. The release of 24OHC from neurons induces ApoE secretion by astrocytes, and both are related to the intensity of the neurodegenerative process and neuronal injury. ApoE can also be involved in the scavenging of tau from neurons. The direct correlations between ApoE, 24OHC and tau suggest that cholesterol metabolism may be involved in generation of both tau and amyloid β-peptide and that the ApoE is released by astrocytes in order to counteract this ongoing process.

Introduction

AD (Alzheimer’s disease) is the most common form of dementia, characterized by degeneration of the neurons and their synapses, extracellular deposition of Aβ (amyloid β-peptide) as senile plaques and intracellular accumulation of aggregated p-tau (hyperphosphorylated tau) as NFTs (neurofibrillary tangles) [1]. Increased production and/or deposition of Aβ is thought to precede tangle formation, but evidence suggests that tau pathology is more closely related to neuronal death [2]. High plasma total cholesterol at midlife is a risk factor of subsequent AD [3], together with the ε4 allele of ApoE (apolipoprotein E) [4] and age [1]. Disturbances in brain cholesterol metabolism have been associated with the major pathological features of AD: synaptic degeneration, amyloid and tau pathology [3–6].

Brain cholesterol metabolism

Cholesterol is involved in cell structure and function. It is an essential component of the cell membranes required for membrane lipid organization and it regulates the fluidity of membranes and the structural disposition of membrane proteins. The human brain contains approx. 25% of the total body cholesterol; approx. 70% is present in myelin, which acts as a discontinuous electrical insulator and increases the speed of electrical depolarization of axons, approx. 20% is present in glia cells, especially astrocytes and microglia, and finally approx. 10% is located in neurons. Cholesterol is also involved in the maturation of the CNS (central nervous system) and participates in signal transduction, neurotransmitter release, synaptogenesis and membrane trafficking [7,8].

Since the BBB (blood–brain barrier) efficiently prevents cholesterol uptake from the circulation into the brain, de novo synthesis is responsible for almost all cholesterol present in this organ.

Neuronal cells have been found to be able to synthesize cholesterol during embryogenesis and early life, but the rate of synthesis falls to a very reduced level in the adult brain. Astrocytes synthesize approx. 2–3-fold more cholesterol than neuronal cells and fibroblasts. Oligodendrocytes, the cell...
type responsible for myelinization, have an even higher capacity for cholesterol synthesis than astrocytes [7,8]. In the mature brain, it was hypothesized that neurons down-regulate their cholesterol synthesis and rely on delivery of cholesterol from nearby cells such as astrocytes which differentiate postnatally and release cholesterol-rich lipoproteins. The ‘outsourcing’ of cholesterol synthesis may allow neurons to focus on the generation of electrical activity rather than dispense energy on costly cholesterol synthesis, particularly in presynaptic terminals and dendritic spines, which are distant from the soma [7,8]. ApoE is the main lipid carrier proteins in the CNS and is released by astrocytes in order to supply neurons with cholesterol. The transcription of ApoE as well the membrane transporters involved in the lipid transport across membranes [ABC (ATP-binding cassette) A1, ABCG1 and ABCG4] have been found to be regulated by 24OHC [(24S)-hydroxycholesterol] released by neurons, via an LXR (liver X receptor)-dependent mechanism [9].

In order to maintain homoeostasis, cholesterol is converted by metabolically active neurons into the more polar 24OHC by a specific cholesterol 24-hydroxylase [CYP46A1 (cytochrome P450 46A1)] expressed in neurons. It has been calculated that approx. 6–8 mg/day of 24OHC is released by the brain into the circulation [10]. A further ∼1–2 mg/day is postulated to leave the brain in some other form, perhaps transported by ApoE [7,11]. CYP46A1 mRNA was found to be mainly expressed in neurons of the cerebral cortex, hippocampus, dentate gyrus, amygdale, putamen and thalamus, i.e. associated with grey matter [12].

**Cholesterol metabolism and Aβ formation**

Cholesterol has been shown to influence a number of processes involved in the generation of the neuritic plaques and NFTs [5,6,13]. APP (amyloid precursor protein) is a transmembrane protein with a large extracellular domain and one transmembrane region. APP can undergo an initial α- or β-cleavage, followed by γ-cleavage, to yield αAPP and P3 peptides or βAPP and Aβ respectively. Increased cholesterol levels and especially increased membrane cholesterol induce the activity of the β-secretase pathway, leading to an accumulation of Aβ1–40 and Aβ1–42 peptides, with a resulting increase in formation of extracellular amyloid deposits [13–15]. Moreover, it has been demonstrated that cholesterol is capable of modulating the production of mature glycosylated APP [15a]. Cholesterol has also been suggested to alter the conformation of Aβ, leading to the generation of ‘amyloid seeds’, regarded to promote the formation of amyloid fibrils from Aβ [16]. Brown et al. [17] showed that 24OHC is an efficient inhibitor of the formation of Aβ under in vitro conditions. 27OHC (27-hydroxycholesterol), which originates outside the brain, seems to be able to cross the BBB, and it was found to have a much lower capacity to inhibit the reaction. 24OHC was decreased and 27OHC increased in brain samples from AD patients [18]. If the oxysterols are important for the generation of Aβ also under in vivo conditions, the reduced levels of 24OHC may accelerate the progress of the disease and the increased levels of 27OHC may not be able to compensate for this. Because of the close relationship between plasma cholesterol and plasma 27OHC [18], the flux of 27OHC over the BBB is likely to increase under conditions of hypercholesterolaemia.

Aβ accumulation, oligomerization and deposition in the brain are central events in the pathogenesis of AD. Its accumulation could reflect overproduction, inefficient clearance or both. ApoE plays an important role in both of the processes. As the main extracellular lipids and cholesterol lipid transporter in the brain, ApoE mediates cholesterol exchange between neuronal and non-neuronal cells. ApoE may also play a crucial role in lipid clearance and recycling, particularly after injury [19–21]. The mechanisms by which ApoE affects AD in an isoform-specific manner have been extensively studied. It was found, for example, that the ApoE4 isoform is less efficient than ApoE3 in facilitating brain function. Supporters of the concept that ApoE has neuroprotective and neurotrophic functions in the normal aging brain argue that ApoE2 and ApoE3 perform these functions more efficiently than does ApoE4 [19–21]. Neurodegeneration in AD may result from impaired delivery of cholesterol from astrocytes to neurons. This impaired delivery would be accentuated by the presence of Aβ and the expression of the ApoE4 isoform [19–21]. The isoform-specific effect of ApoE in AD may be explained by the differential ability of ApoE3 and ApoE4 to supply cholesterol to neurons after injury [22]. Also, a lower efficiency of ApoE4 to stimulate ABCA1-dependent cholesterol efflux [23–25] was found compared with ApoE3, and, finally, ApoE4 seems to promote aggregation of Aβ (more efficiently than ApoE3 or ApoE2) into irregular protofilament-like structures that are neurotoxic to a cultured mouse neuronal cell line [15a]. Additionally, ApoE4 has a greater tendency than ApoE3 or ApoE2 to promote Aβ aggregation in vitro and amyloid plaque deposition in vivo [19–21].

**Brain cholesterol and tau protein**

Recently, it was found that mature hippocampal neurons were more susceptible to Aβ-induced generation of the neurotoxic 17 kDa tau fragment than younger ones. Also, the amount of membrane cholesterol plays an active role in this calpain-dependent mechanism [26]. According to the tau hypothesis of neurodegeneration, the aggregation of tau into filaments and NFTs results in a toxic gain of function. In AD, the number of NFTs in the neocortex positively correlates with the severity of cognitive decline [27]. Thus it is assumed that NFTs are directly able to induce neuronal damage. Because high concentrations of tau are required to promote tau fibrilization in experimental models, it is believed that the enhanced ability of tau to form filamentous inclusions in the cytoplasm of neurons and glia in human tauopathies may be due to pathological conditions that increase the pool of tau available for aggregation. Elevated levels of free tau, not bound to microtubules, would presumably enhance
the assembly of tau into oligomers and could increase its likelihood of becoming misfolded, as well as undergoing modifications or conformational changes that promote the formation of insoluble filamentous inclusions. Tau hyperphosphorylation is an early event in the pathogenesis of tauopathies, appearing before the development of NFTs [28]. In AD-affected brains, the levels of total tau are approx. 8-fold higher than in age-matched controls, together with higher levels of abnormal p-tau. Elevated levels of p-tau are also detected in the CSF of AD patients and may be predictive of neurodegeneration [29,30]. It was found that transgenic overexpression of ApoE4 in neurons, but not in astrocytes, increases tau phosphorylation in mice, suggesting a neuron-specific effect of ApoE4 on tau phosphorylation. The pathophysiological significance of this pathway is not clear, as ApoE is generally produced by astrocytes and microglia, not neurons. However, ApoE expression in neurons has been reported after injury [19–21]. To summarize, both cholesterol and ApoE seem to be involved directly in the process of Aβ formation, and in an indirect way in the process of tau phosphorylation, aggregation and deposition. Finally, both ApoE and cholesterol mediate the toxic effect of such proteins.

**ApoE, 24OHC and tau in CSF**

In several neurodegenerative diseases such as multiple sclerosis and AD, we demonstrated a significant increase in CSF levels of 24OHC [19,20,31–34]. In the case of AD, we found that it was increased in MCI (mild cognitive impairment) and AD compared with controls, together with tau and p-tau [34]. As expected, since the Aβ1–42 tends to be reduced in AD patients, this pattern was significantly reversed. Similarly, the CSF levels of ApoE were increased compared with controls and we found significant correlation between the CSF levels of ApoE, cholesterol, 24OHC and tau (and p-tau), levels which tend to increase with the worsening of the clinical profile of dementia in a significant pattern [34]. Cholesterol accumulation or impaired distribution of cholesterol has been found to be toxic for the neuronal cells [23]. Neuronal damage is accompanied by destruction of neuronal membranes, with an increase in intracellular and membrane cholesterol. In order to maintain homeostasis, the excess of cholesterol is converted by neurons into 24OHC or effluxed to HDL (high-density lipoprotein)-like ApoE lipoproteins via the ABCA1 and ABCG1 transporters located on neuronal cell membranes [35]. Low concentrations of 24OHC released by neurons are able to stimulate neighbouring astrocytes to synthesize ApoE discs which subsequently promote the efflux of non-modified cholesterol. The neuronal cholesterol efflux plays also a significant role in the suppression of APP processing and generation of Aβ. According to this hypothesis, after injury of the CNS, there is a dramatic up-regulation of ApoE that parallels cholesterol and lipid clearance from the site of injury [19–21].

Tau pathology is closely related to neuronal injury and death [28]. Increased CSF tau and p-tau levels are considered to be markers of neuronal injury, which correlates well with the NFT stage and NFT load [30,36]. Atrophy on structural MRI (magnetic resonance imaging) also correlates with Braak’s NFT stages and NFT load, but the most proximate histological correlate of MRI volume loss is loss of neuron and synapses [30,36]. Finally, tau was found to be correlated with the cognitive decline [28].

We observed a significant increase in CSF ApoE and 24OHC correlated to the levels of tau. As ApoE is the main lipid carrier and is also involved in the cholesterol efflux from neurons, it seems that 24OHC acts as a signal to the astrocytes, in order to up-regulate the expression and secretion of ApoE to reduce neuronal cholesterol.

We can speculate that there is a quantitative relationship between the amount of neuronal cells involved and the amount of cholesterol oxidized into 24OHC and the amount of ApoE released. With this perspective, it is interesting that we found both ApoE and tau to be correlated with the amount of grey matter and parenchyma in the brain (V. Leoni, A. Solomon, M. Kivipelto and I. Björkhem, unpublished work). In view of the recently discovered role of membrane cholesterol in Aβ-mediated tau toxicity [26], it is likely that the induction of ApoE expression by 24OHC is a protective mechanism of neurons during the process leading to their injury and degeneration in order to maintain membrane and cellular cholesterol levels, and, together in a direct or mediated way, also the formation of Aβ and tau. The release of 24OHC by neurons plays a signalling role for cholesterol homeostasis in the adult brain, a pathway which is involved in AD. First, 24OHC induces, via LXR, the expression and release of ApoE and cholesterol from the astrocytes for neurotrophism. Secondly, it maintains control of the intracellular amount of cholesterol via hydroxylation or via efflux to ApoE particles. Thirdly, it directly reduces Aβ formation and indirectly Aβ scavenging via ApoE [19–21]. Fourthly, the induction of ApoE expression contributes to the modulation of tau pathology via the reduction of neuronal cholesterol.

In our investigations, we also found that the proportion of ApoE4 carriers is higher in AD patients compared with MCI and controls in a significant positive trend which is correlated with the aforementioned progressive increase in 24OHC, ApoE and tau observed in the CSF.

This positive correlation can be explained by the fact that the proportion of AD-affected individuals is significantly higher compared with the control population, and they have higher levels of ApoE, 24OHC and tau (and p-tau). Anyway, ApoE4 is less efficient in delivering cholesterol to the neurons as well as less efficient in removing it by these cells, and increased generation and poor clearance of Aβ in AD was found to be associated with ApoE4. Thus it is likely that the mechanisms leading to AD are less efficiently counteracted by ApoE4 in the ε4 allele carriers, resulting in higher levels of the mediators of the process we described above.
CSF levels of tau and p-tau, together with Aβ1-42, are commonly used for the diagnosis of AD [1]. In view of our observations, we think that 24OHC and ApoE should also be added to the panel of biomarkers for the study of AD in order to monitor brain cholesterol metabolism in these patients.

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