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Association between acetylcholinesterase and β-amyloid peptide in Alzheimer's cerebrospinal fluid

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ABSTRACT

The altered expression of acetylcholinesterase (AChE) in the brains of patients with Alzheimer's disease (AD) has raised much interest of late. Despite an overall decrease in the AD brain, the activity of AChE increases around β-amyloid plaques and indeed, the β-amyloid peptide (Aβ) can influence AChE levels. Such evidence stimulated our interest in the possibility that the levels of AChE and amyloid might vary together in AD. We previously found that the different AChE forms present in both the brain and in the cerebrospinal fluid (CSF) of AD patients varied in conjunction with abnormal glycosylation. Thus, the alterations in glycosylation are correlated with the accumulation of a minor subspecies of AChE monomers. We also recently analysed whether long-term exposure to the cholinesterase inhibitor (ChE-I) donepezil influences the AChE species found in AD CSF. The marked increase in CSF-AChE activity in AD patients following long-term treatment with donepezil was not paralleled by a rise in this subset of light variants. Hence, the correlation with the levels of CSF-Aβ is unique to these AChE species in patients receiving such treatment. The aim of this report is to review the links between AChE and β-amyloid, and to discuss the significance of the responses of the distinct AChE species to ChE-I during the treatment of AD.

1. Introduction

Alzheimer's disease (AD) is an age-associated neurodegenerative disorder characterized by progressive loss of memory and cognition. The formation of β-amyloid plaques and neurofibrillary tangles are the main neuropathological changes associated with AD, coupled with neuronal loss or dysfunction [1]. Amyloid plaques contain a major protein known as the β-amyloid protein (or Aβ), a polypeptide generated by processing of the much larger amyloid precursor protein (APP; [2]). However, a minor C-terminal form of the Aβ peptide, Aβ42, has since been shown to aggregate more rapidly than the major Aβ40 form [3] and are the predominant species of Aβ in senile plaques [4]. As a result Aβ42 has become a better biomarker for AD than total Aβ [5].

Another significant factor in AD is the loss of forebrain cholinergic neurons and the pronounced loss of acetylcholine [6,7]. The decrease in cholinergic activity affects the activity of the acetylcholine-synthesizing enzyme, choline acetyltransferase, but also that of the acetylcholine-hydrolyzing enzyme, acetylcholinesterase (AChE, E.C. 3.1.1.7; 6, 7). Thus, and due to the critical role for acetylcholine in cognitive function, therapies designed to reverse the cholinergic deficit characteristic of AD are based on the reversible inactivation of AChE. However, although cholinesterase inhibitors (ChE-I) have proven to be effective in treating the cognitive and functional symptoms associated with AD [8], the therapeutic effect of this class of drugs

Abbreviations: Aβ, β-amyloid peptide; AChE, acetylcholinesterase; AD, Alzheimer's disease; APP, amyloid precursor protein; ChE-I, cholinesterase inhibitor; CSF, cerebrospinal fluid.

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is modest. Indeed, not all patients respond equally to these drugs and the cognitive benefits have a limited duration [9].

It should also be born in mind that ChE-I will inhibit cholinesterase activity throughout the brain, indiscriminately affecting AChE species that may have specific physiological functions beyond its role in neurotransmission. Therefore, additional information regarding the biochemical effects of long-term ChE-I treatment on the different species of AChE is needed. Indeed, AChE expression is markedly altered in the AD brain and several studies have considered the existence of a strict relationship between amyloid processing and AChE activity. For this reason, the study of AChE in AD is still of interest. The purpose of this article is to review the links between AChE and one of the main hallmarks of AD pathology, β-amyloid, summarizing recent findings. These include the evidence that AChE levels are positively correlated with Aβ42 levels in AD cerebrospinal fluid (CSF) and that the diverse AChE species differ in their response to long-term treatment with the ChE-I donepezil.

2. AChE and AD

Much interest has been generated regarding the altered expression of AChE in the AD brain. A number of studies, notably those carried out by Mesulam et al., have demonstrated that despite the overall decrease in AChE activity in the AD brain, the levels of this enzyme increases around the amyloid plaques and in tangle-bearing neurons [10–12]. Interestingly, the activity of AChE associated with neuritic plaques in the AD brain displays particular enzymatic properties and sensitivity to inhibitors [13,14]. The association between AChE and amyloid has yet to be explained, but these findings raise the possibility that β-amyloid may influence the expression of AChE.

Conversely, AChE may play a role in Aβ fibrillogenesis. Indeed the incorporation of AChE as a 'chaperone' into β-amyloid aggregates in vitro, augments fibril assembly and increases Aβ neurotoxicity [15]. The peripheral anionic site of the enzyme has been implicated in the nucleation of amyloid peptides [15]. The demonstration that AChE can promote and accelerate the deposition of amyloid plaques in vivo was generated by crossing APP and AChE transgenic mice. Plaque onset in the double transgenic animals occurred sooner and more plaques were formed than in the parental APP line [16]. Several authors have also suggested that ChE-I could affect the expression and metabolism of APP [17–19], particularly given that cholinergic mechanisms can modulate amyloid metabolism [20–22].

Finally, the significance of the different species of AChE is also intriguing, as their specific subcellular distribution is thought to reflect the specific physiological functions of each form. Thus, as well as the different molecular forms of AChE fulfilling different roles, they are likely to have different regulatory requirements. The complex structural polymorphism of AChE produces patterns of molecular forms that depend on the developmental state [23] and the forms altered in AD [15,24,25]. It has been shown that the major AChE tetramers (G_4) decrease in the AD brain, whereas there is a minimal change in the minor light forms (G_1 monomers and G_2 dimers). Furthermore, increases in the levels of the monomeric forms have been described in the vicinity of amyloid plaques [26].

3. Aβ-peptides induce changes in AChE levels and in particular of G_1 AChE

We and others have demonstrated that Aβ peptides influence AChE in vitro, increasing its activity in several neuroblastoma cell lines [27,28], retinal cells [29] and in primary cultures of cortical neurons and astrocytes [30]. We also found that there is more salt-extractable amphiphilic monomeric AChE in the brains of transgenic mice that produce human Aβ [31]. Indeed, G_1 AChE levels are also increased in both the cortex and CSF of rats that received intracerebroventricular injections of Aβ25-35 [32]. As indicated above, the proportion of this particular monomeric AChE specie augments in the AD brain although to date, the significance of this increase for AD pathogenesis is unclear.

4. A small subset of G_1 AChE is abnormally glycosylated in the AD brain and CSF

Sedimentation analysis cannot distinguish between monomeric isoforms that are: (a) synthesized for assembly into oligomers or that arise through degradation; or (b) those that represent specific monomeric variants with potentially specific functions. The human AChE subunit contains three potential N-linked glycosylation sites [33] and it is initially N-glycosylated in the endoplasmic reticulum before carbohydrate moieties are modified in the Golgi apparatus [34]. Due to the important role of glycans in folding, intracellular trafficking, localization and the function of glycoproteins, it is not surprising that there are differences in the glycosylation of the molecular forms of AChE from distinct tissues [35], and even from the same tissue [36]. The ability of lectins to recognize specific carbohydrates in glycoproteins, thereby detecting subtle differences in glycosylation, makes them excellent tools to investigate pathological changes in glycosylation.

Accordingly, we examined the lectin binding in our samples to further characterize different subsets of AChE molecular isoforms. We identified an alteration in the glycosylation of AChE that occurs in the brain [25], post mortem CSF [37] and lumbar CSF collected ante-mortem [38] from AD patients. We characterized that its unusual glycosylation pattern prevents it from binding to the lectin concanavalin A (Con A). This change in AChE glycosylation augments as the disease progresses [39]. We demonstrated that the altered glycosylation is paralleled by a depletion in the tetrameric G_4 molecular form, as a consequence of neuronal loss, as well as an increase in a minor subset of the G_1 species, possibly due to altered Aβ metabolism (see Fig. 1). The contribution of this minor G_1 subset increases in the AD brain and CSF [25,38,40], and in transgenic mice models [31]. Although the exact nature of this G_1 glycoform remains to be established, this minor G_1 specie can be distinguished from other brain AChE forms, both major tetramers as well as other monomeric AChE isoforms, as it is not recognised by anti-AChE monoclonal antibodies (clone HR2 and AE1; [41,42]).