Journal of the Neurological Sciences xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Immunotherapy against amyloid pathology in Alzheimer's disease

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ARTICLE INFO

Article history:
Received 29 September 2012
Received in revised form 9 November 2012
Accepted 10 December 2012
Available online xxxx

Keywords:
Alzheimer's disease
Amyloid
Disease-modifying drugs
Vaccination
Passive immunization

ABSTRACT

The first drugs developed for Alzheimer's disease (AD), anticholinesterase inhibitors (AchEI), increase acetylcholine levels, previously demonstrated to be reduced in AD. To date, four AchEI are approved for the treatment of mild to moderate AD. A further therapeutic option available for moderate to severe AD is memantine. These treatments are symptomatic, whereas drugs under development are supposed to modify pathological steps leading to AD, thus acting on the evolution of the disease. For this reason they are currently termed "disease modifying" drugs. To block the progression of the disease, they have to interfere with pathogenic steps at the basis of clinical symptoms, including the deposition of extracellular amyloid beta (Aβ) plaques and of intracellular neurofibrillary tangles. The most innovative approach is represented by the vaccination and passive immunization against Aβ peptide.
In this article, current knowledge about concluded and ongoing clinical trials with both vaccination with different antigens and passive immunization will be reviewed and discussed.

1. Introduction

1.1. Pathogenesis of Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, with a prevalence of 5% after 65 years of age, increasing to about 30% in people aged 85 years or older. It is characterized clinically by progressive cognitive impairment, including impaired judgment, decision-making and orientation, often accompanied, in later stages, by psychobehavioral disturbances as well as language impairment. Mutations in genes encoding for amyloid precursors protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) account for about 5% of cases, characterized by an early onset (before 65 years of age). So far, 33 different mutations, causing amino acid changes in putative sites for the cleavage of the protein, have been described in the *APP* gene in 90 families, together with 185 mutations in *PSEN1* (in 405 families) and 13 in *PSEN2* in 22 families (http://www.molgen.vib-ua.be).

The two major neuropathology hallmarks of AD are extracellular amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). The production of Aβ, which represents a crucial step in AD pathogenesis, is the result of cleavage of APP, which is over-expressed in AD [1]. Aβ forms highly insoluble and proteolysis resistant fibrils known as senile plaques (SP). NFTs are composed of the tau protein. In healthy subjects, tau is a component of microtubules, which represent the internal support structures for the transport of nutrients, vesicles, mitochondria and chromosomes within the cell. Microtubules also stabilize growing axons, which are necessary for the development and growth of neurites [1]. In AD, tau protein is abnormally hyperphosphorylated and forms insoluble fibrils, originating deposits within the cell.

A number of additional pathogenic mechanisms, possibly overlapping with Aβ plaque and NFT formation, have been described, including inflammation [2] oxidative damage [3], iron deregulation [4], cholesterol metabolism [5].

2. Symptomatic treatments

Acetylcholine levels were demonstrated to be reduced in AD [6]. Based on this observation, the first drugs developed for AD were aimed to increase the levels of such neurotransmitter by inhibiting cholinesterase activity, and were named anticholinesterase inhibitors (AchEI). At present, four AchEI are approved for the treatment of mild to moderate AD: tacrine (First Horizon Pharmaceuticals), donepezil (Pfizer), rivastigmine (Novartis), and galantamine (Janssen) [7,8]. Donepezil is now approved also for severe AD. Although tacrine was the first drug approved for AD in 1993, it is rarely used due to hepatotoxicity.

A meta-analysis of thirteen randomized, double blind, placebo controlled trials with donepezil, rivastigmine and galantamine were considered by the Cochrane Dementia and Cognitive Improvement Group's Specialized Register. Conclusions were that the three AChEI are efficacious for mild to moderate AD, although it is not possible to identify patients who will respond to treatment prior to treatment.

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http://dx.doi.org/10.1016/j.jns.2012.12.013

There is no evidence that treatment with an AChEI is not cost effective. In addition, despite the slight variations in the mode of action of the three AChEI, there is no evidence of any differences among them with respect to efficacy. There appears to be less adverse effects associated with donepezil compared with rivastigmine. It may be that galantamine and rivastigmine match donepezil in tolerability if a careful and gradual titration routine over more than 3 months is used. Titration with donepezil is more straightforward and the lower dose may be worthy of consideration [9].

Recently, it was demonstrated that continued treatment with donepezil is associated with cognitive benefits that exceeded the minimum clinically important difference and with significant functional benefits over the course of 12 months in patients with moderate or severe AD [10].

Rivastigmine is currently available also as a transdermal patch (Exelon® patch, Rivastach® patch, Prometax® patch). Previous evidence suggests that rivastigmine transdermal patch is an effective treatment option for patients with AD, with the potential for improving compliance and providing sustained clinical benefit because of its ease of use and generally favorable tolerability profile as compared with oral AchEI (see [11] for review).

A further symptomatic therapeutic option available for moderate to severe AD is memantine. This drug is an un-competitive, moderate-affinity, NMDA antagonist believed to protect neurons from excitotoxicity. A recent meta-analysis on the efficacy of AChEIs and memantine indicates that these treatments can result in statistically significant but clinically marginal improvement [12]. Regarding tolerability, AChEIs are associated with cholinomimetic effects. Nausea (2–8%) and vomiting (1–5%) were reported across all AChEI trials as the most common reasons for trial discontinuation. Dizziness, anorexia, and diarrhea were also commonly experienced; however, improved tolerability has been reached with transdermal administration of rivastigmine. The most frequently reported adverse events in memantine trials were dizziness, headache, and confusion [13].

3. Modulation of amyloid deposition through vaccination

On the basis of recent additional findings on AD pathogenesis, novel treatments under development aim to interfere with pathogenic steps previously mentioned, in an attempt to block the course of the disease in early phases (even preclinical). For this reason they are currently termed “disease modifying” drugs. As it is thought that the amyloid deposition is the very first pathogenic event in AD pathogenesis, that in turn activates a cascade of additional pathogenic mechanisms, the majority of new approaches under development aim to avoid Aβ peptide deposition or to remove already deposited amyloid. In this framework, the most studied and promising approach is immunotherapy.

3.1. The amyloid hypothesis

The APP plays a central role in AD pathogenesis and in AD research, as it is the precursor of Aβ, which is the heart of the amyloid cascade hypothesis of AD.

The human *APP* gene was first identified in 1987 by several laboratories independently [14–16]. The two *APP* homologs, *APLP1* and *APLP2*, were discovered several years later. APP is a type I membrane protein. Two predicted cleavages, one in the extracellular domain (β-secretase cleavage) and another in the transmembrane region (γ-secretase cleavage) are necessary to release Aβ from the precursor protein. Remarkably, *APP* lies in chromosome 21, and this provided an immediate connection to the invariant development of AD pathology in trisomy 21 (Down's syndrome). The first mutations demonstrated to be causative of inherited forms of familial AD were identified in the *APP* gene, providing evidence that APP plays a central role in AD pathogenesis. Notably, only *APP* but not its homologs *APLP1* and *APLP2*, contains sequences encoding the Aβ domain.

Full-length APP undergoes sequential proteolytic processing. It is first cleaved by α-secretase (non-amyloidogenic pathway) or β-secretase (amyloidogenic pathway) within the luminal domain, resulting in the shedding of nearly the entire ectodomain and generation of β- or β–C-terminal fragments (CTFs). The major neuronal β-secretase, named BACE1 (β-site APP cleaving enzyme), is a transmembrane aspartyl protease which cleaves APP within the ectodomain, generating the N-terminus of Aβ [17]. Several zinc metalloproteinase, and the aspartyl protease BACE2, can cleave APP at the β-secretase site [18] localized within the Aβ domain, thus hampering the generation of intact Aβ.

The second proteolytic event in APP processing involves intramembranous cleavage of α- and β-CTFs by γ-secretase, which liberates a 3 kDa protein, named p3, and Aβ peptide, into the extracellular milieu. The minimal components of γ-secretase include presenilin PS1 or PS2, nicastrin, APH-1 and PEN-2 [19]. Biochemical evidence is consistent with PS1 (or PS2) as the catalytic subunit of the γ-secretase, whereas APH-1 and PEN-2 stabilize the γ-secretase complex, and nicastrin mediates the recruitment of APP CTFs to the catalytic site of the γ-secretase. Major sites of γ-secretase cleavage correspond to positions 40 and 42, leading to the formation of the Aβ[1–40] and Aβ[1–42] peptides.

Amyloidogenic processing is the favored pathway of APP metabolism in neurons, due to the greater abundance of BACE1, whereas non-amyloidogenic pathway predominates in other cell types.

It seems that none of the above mentioned secretases have unique substrate specificity towards APP. Besides APP, a number of other transmembrane proteins undergo ectodomain shedding by enzymes with α-secretase activity. Regarding BACE1, its low affinity for APP led to the hypothesis that APP is not its sole physiological substrate. Similarly, PS1 and PS2 play a crucial role in intramembranous γ-secretase cleavage of several type I membrane proteins other than APP, including Notch1 receptors and its ligands [20].

A number of functional domains have been mapped to the extra- and intracellular region of APP, including metal (copper and zinc) binding, extracellular matrix components (heparin, collagen and laminin), and neurotrophic and adhesion domains. Thus far, a thropic role for APP has been suggested, as it stimulates neurite outgrowth in a variety of experimental settings. The N-terminal heparin-binding domain of APP also stimulates neurite outgrowth and promotes synaptogenesis [21].

APP was initially proposed to act as a cell surface receptor. Nevertheless, the evidence supporting this hypothesis has been unconvincing. In 2004, a candidate ligand had been proposed. In was in fact reported that F-spondin, a neuronal secreted signaling glycoprotein that may function in neuronal development and repair, binds to the extracellular domain of APP as well as of APLP1 and APLP2 [22]. This binding reduces β-secretase cleavage of APP, suggesting therefore that F-spondin binding may regulate APP processing.

3.2. Vaccination

In 1999 Schenk et al. [23] demonstrated that immunization with Aβ as an antigen attenuated AD-like pathology in transgenic mice over-expressing the *APP* gene, by removing amyloid from the central nervous system. This transgenic mouse model of AD progressively develops several neuropathological features of the disease in an age-related and brain-region-dependent manner. Immunization of young animals with Aβ prevented the development of plaque formation, neuritic dystrophy and astroglyosis, whereas in older animals, vaccination reduced extent and progression of AD-like pathologies. Given these extremely promising preclinical results, a phase I controlled study was started, in a cohort of 80 patients with mild to moderate AD. Results demonstrated that this vaccine induced an amyloid

Please cite this article as: Galimberti D, et al, Immunotherapy against amyloid pathology in Alzheimer's disease, J Neurol Sci (2013), http://dx.doi.org/10.1016/j.jns.2012.12.013