Role of Antimicrobial Peptides in Alzheimer's Disease

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Alzheimer's disease (AD) represents the most frequent type of dementia in elderly people. There are two major forms of the disease: sporadic (SAD) - whose causes are not completely understood - and familial (FAD) - with clear autosomal dominant inheritance. The two main hallmarks of AD are extracellular deposits of amyloid-beta (Aβ) peptide and intracellular deposits of the hyperphosphorylated form of the tau protein (P-tau). An ever-growing body of research supports the infectious hypothesis of sporadic forms of AD.

antimicrobial peptides (AMPs) Alzheimer's disease (AD) infectious hypothesis lactoferrin defensins cystatins thymosin β4

1. Introduction

Alzheimer's disease (AD) is the most frequent type of dementia in elderly people [1]. The clinical features of AD include both cognitive decline and a set of non-cognitive symptoms involving perception, mood, personality, and basic functioning, overall known as Neuropsychiatric or Behavioral and Psychological Symptoms of Dementia (BPSD) [2]. Two major forms of the disease exist: sporadic (SAD) - whose causes are not completely understood - and familial (FAD) - with a clear autosomal dominant inheritance [3].

2. AMPs Involvement in AD

2.1. Aβ

The idea that Aβ could be considered as a component of the innate immune system was first proposed in 2002 by Robinson and Bishop [4] in the "*Bioflocculant hypothesis*". According to the authors, the aggregative properties of Aβ are due its ability to surround and sequester pathogens in the brain to limit their spread and - at the same time - prepare phagocytosis. This hypothesis was supported by the identification of microbial DNA within Aβ senile plaques and the attraction of the positive charge of Aβ by the negatively charged membrane of pathogens [4]. A few years later, a low production of $A\beta_{1-42}$ and an increased risk of infections in immunocompetent β-secretase knockout mice were documented [5]. In the same manner, an increased rate of infections in AD patients treated with the $A\beta_{1-42}$-lowering agent tarenflurbil has been reported [6]. More directly, Soscia et al.[7] discovered that $A\beta_{1-40}$ and $A\beta_{1-42}$ exert in vitro antimicrobial activity against eight common microorganisms with a potency equivalent to, and in some cases greater than, LL-37. In addition, the authors found an Aβ-mediated activity against yeast in brain

homogenates of AD patients. The antimicrobial properties of Aβ were also confirmed by Spitzer et al. [8] which demonstrated that $Aβ_{x-42}$ variants, but not $Aβ_{x-40}$ variants, can bound to microbial surfaces and induce microbial agglutination. In addition, $Aβ_{x-42}$ killed up to 80% of microorganisms in all tested pathogens (i.e., bacteria and yeast), whereas $Aβ_{1-40}$ only had a moderate anti-yeast activity. To summarize, these results are consistent with the protective Aβ activity as AMP against pathogens that, when dysregulated, could lead to AD pathology.

More specifically than the Bioflocculant Hypothesis [4] and the Amyloid Protection Hypothesis [9], Moir et al. [10] proposed the Aβ "Anti-microbial Protection Hypothesis". In line with the studies above discussed, the authors suggested that Aβ may play a function as an AMP; thus, representing a type of innate immune defense peptide that protects the host against a variety of pathogens. The persistent activation of this pathway could lead to chronic inflammation and neurodegeneration in AD.

In addition to the high concentration of total tau (T-tau) and P-tau, the reduced levels of $Aβ_{1-42}$ represent the third core CSF biomarkers for AD [11], whereas the ability to discriminate AD from non-AD patients based on the blood levels of $Aβ_{1-42}$ remains unclear [12]. Indeed, a recent literature review showed that the salivary level of $Aβ_{1-42}$ could represent a worthy candidate biomarker for the diagnosis of AD [13].

2.2. Lactoferrin

The antimicrobial proprieties of lactoferrin are conferred by its highly positive charged N-terminal region which ensures that it can provide first line of defense against bacteria, viruses, fungi, free radicals, protozoa, and yeasts [14].

Interestingly, lactoferrin has been shown to bind Aβ [15] and detected in high concentration in neurons and glial cells [16], Aβ senile plaques, and neurofibrillary tangles [16] of the AD brains. In particular, Osmand and Switzer [17] found that lactoferrin is a constituent of Aβ senile plaques and neurofibrillary tangles of the limbic system in brain tissues of post-mortem AD. Kawamata et al. [16] extended these results by showing that lactoferrin is highly expressed and upregulated in both neurons and glial cells (astrocytes, oligodendrocytes, and microglia) of the brain tissues of AD patients compared to normal controls. In addition, the authors find that its expression increases with age and colocalizes with Aβ senile plaques and neurofibrillary tangles of nearly all AD-affected areas, most notably the hippocampus, angular cortex, and entorhinal cortex. Despite these promising results, only 16 years later another research group investigated and better characterized the role of lactoferrin in AD [18]. In detail, An et al. [18] analyzed the expression and localization of lactoferrin transcript in the cerebral cortex of AD and normal controls using real-time polymerase chain reaction (RT-PCR) and in situ hybridization. The results showed greater expression of lactoferrin mRNA in the cortical neutrophilic leukocytes of AD patients, compared to the control group. Given that neutrophilic leukocytes are localized in the activated microglia, the increased release of lactoferrin could occur during the inflammatory process in AD.

In light of the infectious hypothesis of AD, these results suggest that, in this pathology, lactoferrin is synthesized and released mainly from activated microglia, in an attempt to counteract the accumulation of Aβ. Intranasal

administration of human lactoferrin in the transgenic mouse model of AD (APPswe/PS1DE9) has been shown to promote the non-amyloidogenic metabolism of APP processing through activation of *α*-secretase and ADAM10, leading to the production of soluble form of APP, sAPPα, having a neuroprotective role. Indeed, sAPPα reduces generation and deposition of Aβ and improves spatial and cognitive learning ability in AD mice [19].

The potential role of lactoferrin in AD treatment has also been tested on human subjects. Fifty AD patients were randomly assigned into two age- and sex-matched groups that received either standard therapy (group 1, AD patients without lactoferrin) or lactoferrin capsules for three months. Results show that the administration of lactoferrin significantly improved cognitive functions, increased the serum levels of acetylcholine, serotonin, antioxidant, and anti-inflammatory markers and the expression of Akt in peripheral blood lymphocytes (PBL), as well as PI3K, and p-Akt levels in PBL lysate. In addition, the treatment with lactoferrin reduces the levels of key players of inflammation and oxidative stress involved in AD pathology (e.g., serum levels of $A\beta_{42}$, cholesterol, oxidative stress markers, IL-6, HSP-90, caspase-3, P-tau, tau, MAPK1, and PTEN) probably modulating the p-Akt/PTEN pathway [20]. Despite these promising results, further studies are needed to confirm and better characterize the efficacy of lactoferrin in the treatment of AD as well as to explore its administration in the prevention of AD.

Beyond treatment, the pioneering studies performed by Carro et al. [21][22] on the Spanish population, suggested that salivary lactoferrin could represent a useful diagnostic tool for AD. In the first study, the authors compared the salivary levels of lactoferrin between amnestic mild cognitive impairment (aMCI) patients (n = 15), AD patients (n = 36), and a cognitively healthy control group (n = 40). Results showed that the salivary lactoferrin levels were significantly reduced in aMCI and AD patients compared with the healthy control group. The decreased lactoferrin concentration was also correlated with MMSE score and the APOE ε4 allele status in patients with aMCI/AD and negatively associated with the stage of disease (aMCI and AD). Using linear regression and ROC analysis, the authors established a cutoff value of 7.43 mg/mL to discriminate aMCI/AD from healthy subjects with a sensitivity and specificity of 100%. This cutoff value was also tested and successfully used to classify another blinded cohort of aMCI, AD, and healthy control subjects. In addition, in a 56-subject AD subcohort the authors found that saliva lactoferrin significantly correlates with CSF $A\beta_{1\text{-}42}$ and CSF T-tau compared to the control group (n = 68). To evaluate whether the reduced concentration of lactoferrin was specific to AD, the authors compared its levels between a cohort of PD subjects (n = 59) and a control group, finding significantly increased levels in the first group. Lastly, the authors also collected evidence on the possibility of predicting the development of aMCI/AD in healthy subjects based on salivary levels of lactoferrin. In particular, they recruited two different cohorts: 116 “nonclinical” and 190 apparently neurologically healthy subjects. Using the previously identified cutoff value, the authors classified 18 subjects with abnormally reduced lactoferrin levels (<7.43 mg/mL) and 288 with normal/high lactoferrin levels (>7.43 mg/mL). From 1 to 5 years later, 14 of 18 subjects had converted to a clinical diagnosis of aMCI or AD, whereas none of the subjects with a negative test value had converted to aMCI or AD. Thus, salivary lactoferrin levels appear to be also a useful tool for early identification of individuals at risk of developing aMCI/AD with a sensitivity of 100% and a specificity of 98.6% and thus more accurately than $A\beta_{1\text{-}42}$ and T-tau in CSF [21]. To better understand whether the decreased salivary lactoferrin levels are specific to AD and thus suitable for its diagnosis, the same research group performed a second study in which the relationship was examined between

salivary lactoferrin and cerebral Aβ load in patients with aMCI, AD, frontotemporal dementia (FTD)—as an example of another type of dementia—and a healthy control group [22]. Data showed that salivary levels were decreased only in aMCI/AD and were associated with amyloid–PET imaging profile; thus, supporting the possible use of this biomarker in the differential diagnosis of AD vs. FDT with a sensitivity and specificity over 87% and 91%, respectively. However, Gleerup et al. [23] attempted to validate the use of salivary lactoferrin to discriminate AD from non-AD patients in the Danish population. In addition, this study was the first to evaluate the diagnostic potential of CSF levels of lactoferrin. Participants were divided into four different groups: healthy subjects (n = 20), MCI (n = 56), AD (n = 71), and non-AD patients (n = 75). The latter group included a heterogeneity of conditions such as vascular dementia (VaD), mixed dementia, FTD, dementia with Lewy bodies (DLB), and Parkinson's disease with dementia (PDD). The results of this study showed that there were no statistically significant differences in the levels of CSF and salivary lactoferrin between the different groups. In addition, no significant relationships were found between lactoferrin and the CFS concentration of well-established dementia biomarkers ($A\beta_{1-42}$, P-tau, and T-tau). However, given the small sample size and the extreme heterogeneity of the control group, it could be useful in future studies to increase the sample and make a comparison between salivary levels of lactoferrin in AD patients and, separately, with other neurodegenerative diseases (e.g., AD vs. FTD vs. VaD vs. DLB vs. PDD). In addition, given its role in iron transport, it would be interesting to investigate whether lactoferrin also plays a role in the well-documented iron accumulation in neurons of AD patients.

2.3. Defensins

Researchers' findings suggest an active role for β-defensin 1 as a potential modulator of the host innate immune response within the central nervous system. Moreover, compared to control people, AD patients show a higher copy numbers polymorphism of the DEFB4 gene - that encodes for β-defensin 4 and influences the production of β-defensin 2 - thus, explaining the increased levels of β-defensin 2 reported in serum and CFS of AD patients [24]. More recently Zhang et al. [25] proposed the "anti-amyloid and antimicrobial hypothesis" of AD which postulates that α-defensins can be considered as multi-target inhibitors to prevent both microbial infection and amyloid aggregation underlying the onset of AD. In support of this hypothesis, the authors found that some α-defensins contain β-rich structures that allow it to cross-interact with Aβ. This binding would seem to prevent the formation of amyloid plaques and to reduce amyloid-induced cell toxicity. Indeed, β-defensins retain their original antimicrobial activity upon the formation of complexes with Aβ.

Although further investigations are needed, these findings open new scenarios for understanding the pathogenesis of AD and underline the therapeutic potential of AMP for amyloid diseases.

2.4. Cystatins

Other evidence supporting the involvement of cystatins in AD derives from genomic studies. It an association between cystatin C gene polymorphism and an increased risk of developing AD has been reported (for a review see: [26]). In addition, a point mutation in the cystatin C gene causes a particularly dominantly inherited type of amyloidosis: the hereditary cystatin C amyloid angiopathy (HCCAA; [27]).

Beyond the possible role of cystatins in the pathogenesis of AD, other studies suggest that they could also be considered good diagnostic biomarkers. Indeed, the levels of cystatin C are reduced in the CFS of AD patients [26], whereas the levels of cystatins A and B are increased in the saliva of AD patients [28].

The potential role of cystatins in the treatment of AD remains largely unexplored. However, preliminary studies indicated that cystatin C appears to be neurotoxic both in vivo and in vitro [29], suggesting that cystatins must be used in future therapeutic studies with a special precaution.

2.5. Thymosin β4

Thymosin β_4 ($T\beta_4$) is a small multifunctional peptide containing 43 amino acids, which protects tissues against damage and promotes their regeneration [30]. It has been reported that in the central nervous system Tβ4 is mainly released by activated microglia to inhibit neuroinflammation [30]; thus, exerting antimicrobial activity [28]. Therefore, it is plausible to hypothesize that in AD Tβ4 may be released by activated microglia—together with other AMPs and other substances—to counteract the inflammation due to the Aβ accumulation. To our knowledge, the possible role of Tβ4 in the pathophysiology of AD has never been investigated and conflicting results have been obtained from the few studies that examined its potential role as a biomarker of AD. Le Pera et al. [31] found unaltered levels Tβ4 in the CFS of AD patients. On the other hand, Contini et al. [28] found increased levels of Tβ4 in the salivary of AD patients compared to a healthy control group. Further studies are needed to better clarify these aspects.

2.6. LL37

LL37 is a cationic and small AMP that belongs to a group of major mammalian AMP named cathelicidin. It is released by several types of cells such as salivary glands, neutrophils, leukocytes [32], as well as neurons and glial cells in response to pathogens [33]. Interestingly, LL37 can also activate astrocytes and microglia to induce the glial-mediated neuroinflammation and thus may exert a role in the pathogenesis of AD [33]. In particular, it has been proposed that neurons, when injured, released LL-37 which in turn activates microglia and astrocytes. Consequently, microglia and astrocytes also release LL-37 which can cause the translocation of NFκB proteins to the nucleus by binding receptors such as FPRL-1, P2 × 7, and P2Y11. In turn, this process can lead to the expression and release of pro-inflammatory cytokines such as TNFα, IL-1, and IL-6, giving rise to a positive feedback mechanism which causes further destruction of neurons [33]. Moreover, in vitro data show that LL37 can bind to $A\beta_{1-42}$ to modulate its ability to form the long and straight fibrils characteristic of AD. Thus, the balanced or unbalanced spatiotemporal expression of $A\beta_{1-42}$ and LL37 could impact AD onset and progression [34]. Recently, it is has been designed using bioinformatics tools as an analog of LL-37, namely kLL-39, that would appear to have an enhanced antimicrobial activity and a reduced toxicity for the host cells [35]. In vitro studies are needed to investigate the antimicrobial effects of kLL-39 in AD.

2.7. Histatin 1 and Statherin

Histatin 1 and statherin represent two salivary peptides that also exert antimicrobial activity [36]. Contini et al. [28] compared the salivary proteome of AD patients with a healthy control group, finding increased levels of these

two AMPs in addition to α-defensins, cystatins A and B in AD patients. Thus, also histatin 1 and statherin can be viewed as interesting objects of interest for future research on AD.

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