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Review

What amyloid ligands can tell us about molecular polymorphism and disease

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ABSTRACT

Brain-penetrant positron emission tomography imaging ligands selective for amyloid pathology in living subjects have sparked a revolution in presymptomatic biomarkers for Alzheimer's disease progression. As additional chemical structures were investigated, the heterogeneity of ligand-binding sites became apparent, as did discrepancies in binding of some ligands between human disease and animal models. These differences and their implications have received little attention. This review discusses the impact of different ligand-binding sites and misfolded protein conformational polymorphism on the interpretation of imaging data acquired with different ligands. Investigation of the differences in binding in animal models may identify pathologic processes informing improvements to these models for more faithful recapitulation of this uniquely human disease. The differential selectivity for binding of particular ligands to different conformational states could potentially be harnessed to better define disease progression and improve the prediction of clinical outcomes.

1. Introduction

Neuropathologists distinguish different types of amyloid-β (Aβ) deposits in histologic sections of brain tissue on the basis of morphological characteristics and staining properties of the lesions. Some have attempted to link diffuse, primitive, classic, and compact Aβ plaques and neurofibrillary (tau) tangles with different stages of Alzheimer's disease (AD) progression. Studies characterizing Aβ deposit ultrastructure, localization, and molecular composition in humans suggest that the different lesion morphotypes are not necessarily developmentally related to one another (Armstrong, 1998, 2009; Thal et al., 2006). Multiple factors influence these features (D'Andrea and Nagele, 2010), and the complexity of morphotype formation in the brain obscures their relationship to pathogenicity and disease progression. Variable rates of disease progression and differences in phenotype of idiopathic AD could result from different relative proportions of the morphotypes. Furthermore, in addition to the classical insoluble fibrillar forms of Aβ and tau, soluble oligomeric forms of the proteins also are present in the AD brain (Bilousova et al., 2016; Catalano et al., 2006; Lue et al., 1999; McLean et al., 1999; Wang et al., 1999). Rapid clinical progression has been connected with a particular Aβ oligomer size distribution, conformational states, and stabilities (Cohen et al., 2015, 2016).

Attempts to understand the in vitro assembly of Aβ and other amyloidogenic proteins and peptides into soluble oligomers and insoluble fibrils at a structural level have revealed a surprising spectrum of polymorphic forms depending on environmental conditions and starting peptide conformation (Eisenberg and Jucker, 2012; Toyama and Weissman, 2011; Tycko, 2015). These assemblies produced in vitro have been structurally characterized at the molecular level by such methods as X-ray crystallography, solid-state nuclear magnetic resonance (ssNMR), and hydrogen-deuterium exchange (Eisenberg and Jucker, 2012; Kodali et al., 2010; Tycko, 2015; Zhang et al., 2009). Because analogous experiments are not feasible on the human AD brain mixture of multimeric Aβ assemblies, fibril-containing brain extracts from 2 separate individuals with AD were used to catalyze multiple rounds of seeded growth of fibrils from stable, isotope-labeled monomeric Aβ. The resultant labeled fibrils were sufficiently homogeneous for

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ssNMR structure to determine that the seeded amyloid fibrils from the 2 AD cases were structurally different (Lu et al., 2013). One interpretation of the findings is that only a different single major conformational type of Aβ was generated in each case or that the distinct fibrillar structures resulted from selection through fitness for in vitro seeding efficiency. Alternatively, structural variation could be the result of selective amplification of the thermodynamically stable conformation, as demonstrated by the changing conformation of the D23N Iowa mutant Aβ through repeated cycles of seeding (Qiang et al., 2011). Selectivity in animal models was maintained during amplification of Aβ pathology obtained from sporadic human AD, Swedish mutation (K670M/N671L), and Arctic mutation (E193G) brain during propagation in theTgAPP23 mouse model (Watts et al., 2014).

Support for the presence of different conformational states of Aβ fibrils in vivo comes from staining tissue sections with poly- and oligothiophene probes that bind to a variety of amyloid-like structures including tau tangles. The spectral characteristics of this class of probes depend on the rigidity of their flexible thiophene backbone (Nilsson et al., 2007). The structural organization of Aβ plaques in the brains of APP/PS1 transgenic mice changes with advancing age, and this transformation is reflected by the binding of thiophene probes with different structures (Nystrom et al., 2013). These observations confirm the polymorphic nature of aggregated Aβ and other amyloid pathology in vivo and indicate that the assemblies undergo age-dependent and perhaps disease stage-dependent structural changes. In this light, it is important to understand the binding characteristics of clinical imaging ligands to polymorphic forms of Aβ and tau pathology to know whether these agents are reporting structures relevant to all stages of AD. This review offers a framework for understanding what structurally different small molecule binding probes can tell us beyond the presence of misfolded proteins and caveats about what they do not provide. We suggest additional ways in which these ligands might be employed to aid in identifying and distinguishing underlying biology, improve diagnosis, and develop/monitor therapeutic interventions.

2. Molecular ligands for systemic amyloid

The development of ligands that selectively bind to misfolded protein pathologies has yielded valuable tools for the in vivo and ex vivo detection and analysis of the lesions. Early studies involved systemic amyloidoses in which misfolded proteins, mainly amyloid A and antibody light chains, deposit in multiple organs such as the kidney, liver, and heart. The prototypical amyloid visualizing ligand is the diazo cotton dye Congo Red (Elghetany et al., 1989). When applied as a histologic stain, the dye accumulates in amyloid deposits in tissue sections and undergoes a metachromatic shift in its absorbance spectrum. Viewed under illumination through crossed polarizing filters, the ordered, β-sheet–rich structure of the amyloid fibrils is evident in the birefringence of the stained deposits (Howie and Brewer, 2009). In one of the first attempts to use a selective amyloid ligand for diagnosis, Bennhold (1923) infused Congo Red intravenously into patients suspected of having a systemic amyloidosis. Rapid disappearance of the dye from the blood was thought to reflect sequestration of the agent in amyloid deposits; unfortunately, the approach had to be abandoned for safety reasons (Buxbaum and Linke, 2012). In the 1980s, the pentraxin serum amyloid P component was introduced as an imaging ligand for systemic amyloidosis (Pepys, 2001). Serum amyloid P has an intrinsic binding capacity for amyloid fibrils; when labeled with either short half-life radioisotopes ^{131}I or ^{123}I for single photon emission computed tomography scintillographic imaging, it is a sensitive and selective tool for detecting amyloid deposits in living patients (Hawkins and Pepys, 1995; Hawkins et al., 1998). The 25-kDa molecular weight of the labeled serum amyloid P limits its use to deposits outside the central nervous system.

3. Ligands for imaging amyloid in the brain

Imaging probes for use in the brain require a combination of molecular properties (molecular weight, hydrophobicity, and resistance to metabolism) to penetrate the blood-brain barrier and reach their targets. Neurodegenerative diseases involving misfolded protein pathology, especially AD, have been a focus of new ligand development because such agents could provide useful information on the presence of disease in a relatively inaccessible body compartment, thereby enabling the early detection and longitudinal monitoring of the pathogenic process (Vlassenko et al., 2012). A multitude of small molecule probes have been prepared and tested for affinity and specificity in vitro, with a much smaller number moving into testing in vivo for binding potential (reviewed in Eckroat et al., 2013). The great majority have been directed at the Aβ peptide or at the microtubule-associated protein tau, which form the pathognomonic lesions of AD: senile (Aβ) plaques and neurofibrillary tangles, respectively. Imaging human subjects with the tau ligand ^{18}F-AV-1451 reveals age-dependent accumulation of tau in regions such as the medial temporal lobe that colocalizes in isocortical regions with Pittsburgh Compound B (PIB) binding to Aβ. The isocortical binding correlates with decline in global cognition. ^{18}F-AV-1451 signal generally recapitulates key features of Braak stage histopathology (Ossenkoppele et al., 2016; Scholl et al., 2016; Schwarz et al., 2016). Targeting other protein aggregates, such as the α-synuclein–containing lesions of Parkinson's disease and Lewy body dementia, has been challenging because of the difficulty in establishing the requisite selectivity to distinguish α-synuclein from the frequently comorbid Aβ and tau misfolded protein pathologies (Neal et al., 2013).

The need for agents to detect amyloid pathology in the brains of living subjects before the onset of the clinical symptoms of AD has driven the search for ligands that can detect and quantify the deposits. The extended conjugated π electron density and aromaticity of the bis-styryl compounds and benzothiazoles confer particularly good histologic selectivity of these compounds for amyloid fibrils. In histologic preparations, the benzothiazoles Thioflavin S and Thioflavin T selectively bind to amyloid deposits of many different proteins and emit a bright fluorescence (LeVine, 1995). Owing to their selectivity and sensitivity, the molecular motifs of agents, such as Congo Red and the Thioflavins, appear in various guises in the structures synthesized as ligands for Aβ deposits in the brain.

Development of high-affinity amyloid-binding ligand structures with appropriate specificity and chemical properties for in vivo imaging and/or inhibition of fibril assembly has been undertaken using a variety of screening paradigms and computational approaches. Progress has been limited by the structural heterogeneity and dynamics of full-length Aβ (1–40) and (1–42) amyloid fibrils. ssNMR structures of the longer peptides have been obtained under certain conditions (Luhrs et al., 2005; Tycko, 2011, 2014). Atomic resolution X-ray crystal structures of short synthetic peptides, in particular Aβ residues KLVFFA, obtained in the absence and presence of small molecule ligands (Landau et al., 2011), have provided detailed structural information. Molecular modeling and dynamics simulation in docking studies gave insight into interactions with small molecule ligands. These methods identified putative binding sites and accounted for differences in affinity between ligands for the model structures (Biancalana and Koide, 2010; Reinke and Gestwicki, 2011; Wu et al., 2007, 2008, 2011). However, experimental determination of the binding affinity and specificity of resulting ligands in human tissue revealed significant disparities