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Alpha-synuclein PET and Parkinson's Disease Therapeutic Trials: Ever the Twain Shall Meet?

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Introduction

Neurodegenerative disorders are common brain afflictions with increasing prevalence rates in countries with aging populations. This is true for the two most common neurodegenerative disorders, Alzheimer's (AD) and Parkinson's (PD), both demonstrating increases in prevalence over the last decade with more than 6 million afflicted with AD and 1 million with PD in the US[1, 2]. The impact on the individual sufferer, the burden on families, and the societal and financial costs are becoming more urgent problems as these epidemiological trends play out[3].

Recently, progress has been made in understanding the etiology of neurodegenerative disorders at the molecular level. We know these diseases represent a series of pathological brain conditions characterized by protein misfolding and the progressive accumulation of cytotoxic fibrils and oligomers that are believed to result in the selective neurological and functional impairment[4]. The unrelenting progression of symptoms and disability represents the ongoing neuronal degeneration resulting from the dissemination of these cytotoxic proteins in brain. In AD there are PET biomarkers targeting the two aberrant proteins, beta amyloid contained in neuritic plaques and tau found in neurofibrillary tangles, the pathology described originally by Alois Alzheimer.

These imaging biomarkers have already been deployed to support the development of therapeutics which target amyloid[5]. The equivalent protein in PD is alpha-synuclein (a-syn) found in Lewy bodies. Currently there is no available PET agent for interrogating a-syn deposition and no easy way to track in vivo brain changes mechanistically relevant to clinical progression[6].

Why Alpha-synuclein PET in Parkinson's

Dopamine transporter (DaT) imaging using single photon emission computed tomography (SPECT) and 123-I tropane imaging agents like 123-I Ioflupane demonstrate significant reductions in striatal binding ratios of PD patients enrolled in longitudinal studies going out 1-2 years or more. DaT SPECT is also an important tool for identifying DaT deficits in premotor and at-risk individuals[7, 8]. Yet DaT imaging interrogates one piece of the pathology of Parkinson's disease, presumably the consequence of alpha synuclein deposition in the substantia nigra. DaT SPECT does not directly assess a-syn, the latter representing an upstream process against which disease- modifying treatments are targeted. Hence the two imaging biomarker targets, DaT and a-syn, are theoretically complementary.

Alpha synuclein (a-syn) is a 140 amino acid protein highly prevalent in the brain representing 1% of the cytosolic protein and thought to be localized to the presynaptic terminal where it facilitates the release of neurotransmitter into the synapse. The protein undergoes extensive posttranslational modification and is natively unstructured, rather taking its conformation from its local milieu. Within cells fibrils form oligomers, thought to be the cytotoxic form of a-syn. Different conformational states characterize the different synucleinopathies[9, 10].

A-syn has now emerged as a key target for development of a PET biomarker in the evaluation of the pathophysiology and putative treatments for modifying the course of Parkinson's disease. Why? First, the primary brain pathology in PD are Lewy neurites and Lewy bodies composed of aggregated a-syn resulting from misfolding of protein and seeding and whose pattern of brain spread is suggested by postmortem brain examination of Lewy body distribution[11, 12]. These studies indicate a discrete pattern of Lewy body formation which describes a pathway of spread going from the brainstem systematically to midbrain (including nigrostriatal projections) and finally to higher cortical regions. A-syn is found in many body tissues outside the brain including the skin, salivary glands, and gut. Biopsy of tissue in PD can demonstrate this and has been proposed as another biomarker in clinical therapeutic trials. Interestingly, recent work suggests the GI tract may have a role in the etiology of Parkinson's disease. Alterations in the microenvironment of the gut biota can lead to inflammatory changes and conditions which favor the formation of a- syn fibrils[13]. These may then travel into the CNS via the vagus nerve and or through vascular pathways. Once seeded in lower brainstem it is hypothesized that fibrils cross the synapse to affect adjacent connected neurons (Figure 1). The presence of these alien fibrils creates conditions that enhance the formation of additional fibrils and aggregates. As a-syn spreads superiorly involvement in midbrain structures may result in some premotor symptoms found to be associated with Parkinson's disease including REM sleep behavior disorder and hyposmia. Both of these have been used for cohort enrichment for enrollment of at-risk or premotor individuals[14].

Another reason for interest in a-syn in Parkinson's disease comes from genetic studies. Genome-wide association studies have shown a strong association of variations in the a-syn gene (SNCA) with PD[15, 16]. In addition, mutations in the SNCA gene promote formation of a- syn aggregates and fibrils. These findings support a-syn as an important target of disease- modifying treatments and potentially a path to better understanding of the onset and longitudinal course of disease across the synucleinopathies; PD, dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Further, knowing how differences in a-syn conformational structure affects pathophysiological manifestations of disease may offer additional clues to treatment, clarify phenotype with regard to differential diagnosis and prognosis, and offer needed tools for conducting therapeutic trials like at-risk screening, proof of target engagement, and assessing drug efficacy.

The enthusiasm for a PET biomarker of a-syn is underscored by the sponsorship of the Michael J Fox Foundation offering a $2 million prize to the first team that develops a viable selective alpha-synuclein PET tracer and agrees to make that tracer available broadly. The ability to image alpha-synuclein deposition in the brain was described in the program announcement as “a game-changing achievement for the Parkinson's disease field”. Efforts by both industry and academic groups are underway in this and other a-syn biomarker initiatives.

New Roles for PET Imaging in Parkinson’s Disease

Up to now treatment for PD has been symptomatic, rather than disease-modifying. Designing a clinical trial of a drug that actually slows down, stops, or reverses clinical symptoms and improves function is exceedingly difficult. Questions arise as to what participants to enroll, what is the metric for measuring treatment efficacy, and how many subjects are necessary to power a potentially small reduction in a process that is changing relatively slowly as progressing Parkinson's?

Medications taken for managing symptoms like L dopa can be another confound in determining off-medication clinical status. To get true off medication assessments several weeks of withdrawal of the symptomatic medications may be necessary. This is not easily done nor ethical given the amount of increased morbidity experienced by patients off medications for that period[17]. Another issue regarding medications is whether the putative disease-modifying agent has any direct symptomatic effect making it harder to tease out symptom relief from true efficacy in altering the mechanisms of disease[18].

Questions about when in the course of illness to recruit the test cohort are extremely important since clinical manifestations occur only after years of silent, abnormal pathologic processes. How does one diagnose and treat a disease that is not clinically manifest? This pre- symptomatic phase of illness is when a disease-modifying intervention might be most effective, rather than later when there is less salvageable tissue. Recruiting from this cohort also gets around the medication issues above-mentioned. The length of this window between initiation of pathology and subsequent clinical manifestation is on the order of years. As example, utilizing the dopamine transporter agent 123-I Ioflupane and SPECT imaging in the Parkinson's Progression Marker Initiative (PPMI) de novo PD cohort[19] serially scanned over 4 years (baseline, years 1,2, and 4) allows back extrapolation of striatal specific binding ratio curves to the point of normalcy permitting a rough estimate of the duration of the clinically silent course of progressive change in the brain.

This turns out to be about 13 years in the most affected brain regions. All this suggests the important roles that a-syn PET might serve in the arena of clinical trials. These include providing an early confirmation of disease pathology in at-risk individuals, serving as a screening tool for ensuring the diagnostic integrity of the cohort, offering a biomarker directly related to the mechanisms of disease progression, providing evidence of target engagement, and assessing the efficacy of an intervention designed to slow down this progression.

Alzheimer’s Drug Development and Tau PET: A model for a-syn PET?

The recent development and application of imaging biomarkers in Alzheimer's disease is a model and reminder of the ways that PET imaging supports therapeutic trials by providing a window on primary pathophysiology. The recent, albeit controversial, FDA approval of the amyloid targeting antibody, aducanumab for AD serves as an example of the integration of PET biomarkers into clinical drug trials and hints to future clinical roles[20]. Perhaps most relevant to the development of a radiotracer for the alpha-synucleinopathies is the recent history of the development of radiotracers for the tauopathies. The parallels between tau and a-syn are significant and could provide a road map of expectations for some potential ways the successful development of an a-syn PET agent might proceed.

Tau PET developed as follows. Briefly, Phase 1 was the period of concept formation, articulation of need, and scientific and medical community buy-in as to the need/desire for targeted tau imaging biomarkers. Next was phase 2, a period of radiochemistry development called the wandering lost in the desert of failed compounds stage. Even so, while wandering research teams were getting more sophisticated about binding affinities and selectivity of candidate structures. The move out of the desert was phase 3 when one or more promising structures were discovered and in vitro and nonclinical evaluation occurred. This led to phase 4 or the human proof of concept trials in AD where there was characterization of the pharmacokinetics and validation of an outcome measure[21]. Phase 5 was the sharing of the pioneering compound amongst investigators and the incorporation into clinical trials as an exploratory outcome. While this was happening further development of second-generation tau tracers was initiated. Finally, Phase 6 was the extensive incorporation of tau tracers as biomarkers in clinical trials and more nuanced understanding of differences in affinity to tau isoforms relative to the use of the radiotracer[22].

Alpha-synuclein PET: Why has it been so difficult?

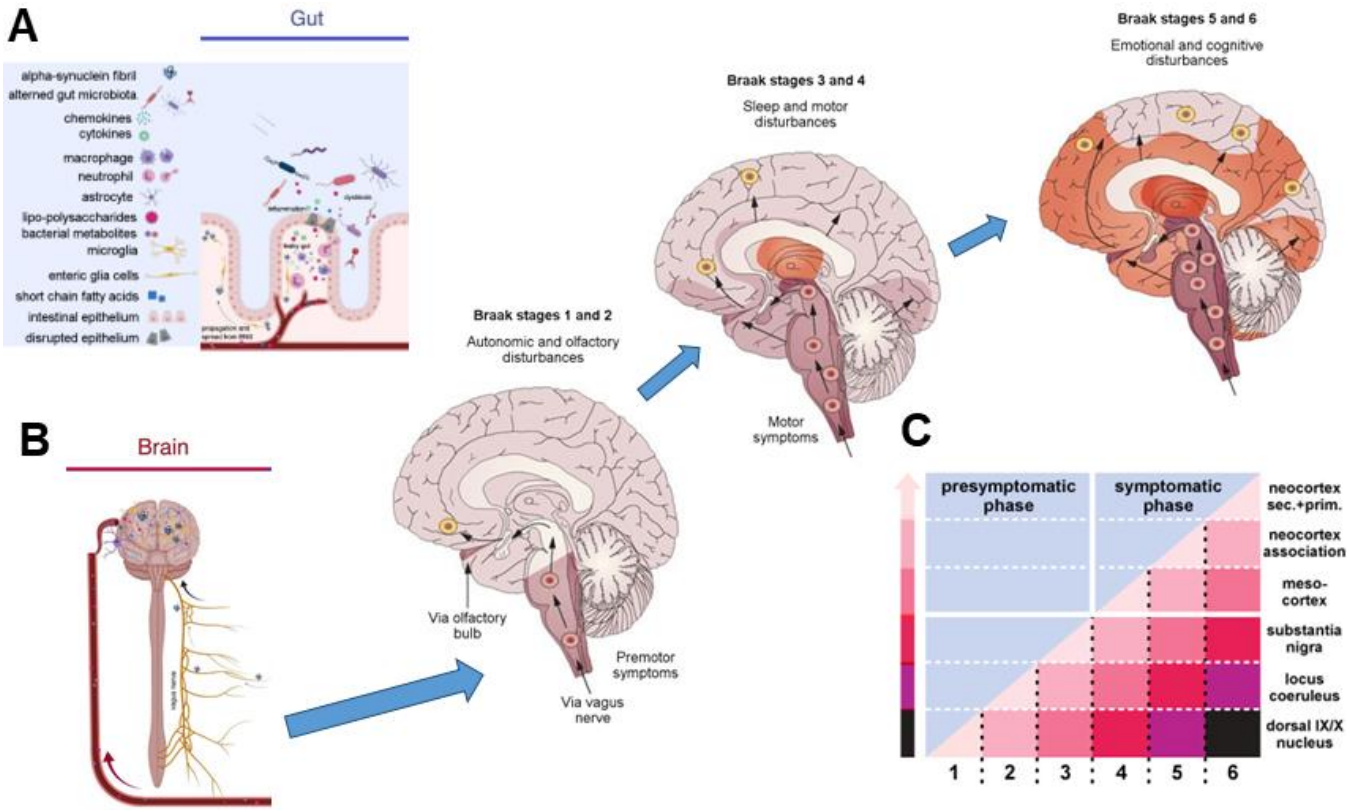
The tau PET experience just considered, why has it been so difficult to develop an a-syn radiotracer for PD? Why not simply label the therapeutic? While compelling, this strategy generally doesn't work out because the properties of a good therapeutic like high lipophilicity for brain penetrance causes higher background and lower PET signal to noise. Further, radiotracers require nanomolar to subnanomolar affinity and high selectivity for a-syn as well a preference for kinetics that offer fast washout of background signal and no confounding labeled metabolites. Fayyad, et al proposed several additional obstacles to developing an a-syn imaging biomarker: no good compound leads (e.g. dyes or tissue stains), lack of a library of compounds for selectivity screening against tau and beta amyloid, low target density, and poor PET resolution[23]. Other issues include the potential confound of isoform heterogeneity and cost. Recent work suggests that the field is addressing these concerns. For example, Ferrie, et al describe an ultrahigh throughput in silico screening strategy using idealized pseudo-ligands (exemplars) to identify compounds, including confirmation of the binding site and evaluation of the structure-activity relationship of analogs for development of multiple molecules with nanomolar affinity for a-syn fibrils[24]. More sophisticated understanding of the conformational-dependent binding sites on a-syn [25] can be used to inform radiotracer (and drug) development [26]. Finally, there have been initial a-syn PET human studies with promising structures, but mixed results to date[27, 28].

Conclusion

Will we ever develop an a-syn PET tracer for clinical trials? Optimistically, there are several factors which suggest we will. There are increasingly pressing needs from the numbers of therapeutic trials with new a-syn targeting treatments. The body of knowledge about a-syn function (binding sites, conformational states, and SAR) and dysfunction (misfolding, aggregate formation, seeding, and spread) inform the medicinal and radiochemistry development as well as the in vivo validation of a-syn PET ligands. In addition, prior experience in AD suggests imaging proteinopathy in PD may be a useful clinical research tool and offers a roadmap for development of a-syn PET. Perhaps most encouraging is the recent presentation (March 2022) of the first apparently successful human a-syn PET agent, AC-12589 from AC-Immune and Oskar Hansson and colleagues at Lund University, Sweden. These very preliminary studies in multiple system atrophy(MSA), PD, and controls demonstrated the expected increased uptake in cerebellar white matter in MSA, but not PD, or healthy volunteers. The lack of specific uptake in PD could be related to a-syn conformational differences with MSA, relative target affinity, small sample size or other factors.

In summary, we already know a great deal about pathological alpha-synuclein formation and spread as well as how to develop and validate imaging tools for clinical and research needs, and even a promising compound in initial human trials. We just need to keep going and make our way through the desert.

Figure 1 An Etiologic Model of PD and other Synucleinopathies



A proposed model for the development of Lewy body disease like PD suggests the gut may be the locus of initial production of a-syn fibrils (A). This occurs in the context of inflammatory changes associated with disruption of the integrity of the intestinal epithelium as occurs in different microbiota environ- ments. These fibrils are taken up by the vagus nerve (B) and transported to the lower brain stem.

Through a process of cell to cell spread following along network lines additional cells become affected systematically moving up through the brainstem to the midbrain and cortex and resulting in progressive symptoms reflective of the involved region (C). Motor symptoms begin at stage 3 when the substantia nigra becomes involved. Adapted from Fitzgerald,et al (2019) and Braak, et al (2003) with permission.

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