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INVITED PERSPECTIVES

The Irony of PET Tau Probe Specificity

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It may be rather surprising that after more than four decades of positron emission tomography (PET) development and thousands of molecular imaging probe candidates synthesized, relatively few among them are consistently used to investigate human diseases while even a much smaller number have reached the domain of patient care. There are very good reasons for this occurrence, however. One of the arguments being put forward focuses on the lack of appropriate synthetic methods for the development of PET biomarkers. However, the history of PET indicates that creative molecular probe design and rational translational evaluation has been the most significant limiting factor.

'Tau-specific' PET imaging probes are no exception to the above rationale. For instance, a F-18 fluorinated analog belonging to a family of 5H-pyrido[4,3-b]indoles, known as T-807, has initially been touted as a tau sensitive and presumably specific PET tau imaging probe. This PET probe candidate was selected from a chemical library of compounds screened primarily using *in vitro* binding to isolated fibrils and macro- and micro-autoradiography procedures (1). Based on these evaluations, T-807 was promptly accepted as a suitable candidate for *in vivo* use with PET with very little additional basic research investigations. The wide availability of this probe, courtesy of Eli Lilly & Co which trademarked it as AV-1451, may bear some responsibility for its hasty utilization to monitor tau deposition using PET in the living brains of Alzheimer's disease (AD) patients.

The connection between tau deposition and neuronal losses clearly provided a good opportunity for a logical approach to investigating disease progression and a possible diagnostic tool for AD using PET, conceptually superior to that resulting from PET monitoring of amyloid aggregates, the other pathognomonic neuroaggregate in AD. Recognition of the significance by the National Institutes of Health soon resulted in generous funding for utilization of AV-1451 in living subjects with AD, but also in other predominant tauopathies, like progressive supranuclear palsy (PSP) and chronic traumatic encephalopathy (CTE), the latter having a three-repeat (3R)/four-repeat (4R) tau isoform ratio similar to that of AD. The broad impact of CTE in society in general, and its close association to the ailments of many of the National Football League (American football) players was also undoubtedly a driving force for this generous extramural support.

Within a short period, AV-1451 became the darling of tau-specific probes, but with diligent PET determinations in living patients, and additional *postmortem* determinations performed, unexpected results and inconsistencies clearly began to emerge. Notable among them were non-specific, tau-unrelated binding in the choroid plexus and striatum, and low sensitivity for distinguishing AD cases from healthy controls (2). Also, while AV-1451 was found to bind to the 3R/4R tau isoforms present in AD, 3R and 4R tau isoforms typically forming straight filaments in predominant tauopathies such as PSP, MAPT P301L mutation carriers, corticobasal degeneration (CBD), and frontotemporal dementia did not seem to be recognized by this PET biomarker (3). The same investigators had earlier reported (4) that autoradiography failed to show detectable AV-1451 binding in multiple brain regions examined *postmortem* in non-AD tauopathies and, further, they also confirmed the presence of non-specific binding of AV-1415, among others, to neuromelanin and melanin-containing cells in the brain, as well as to brain hemorrhagic lesions.

Another comprehensive report (5) assessing AV-1451 binding to *postmortem* brain tissue from patients with a range of neurodegenerative diseases, tauopathies and non tauopathies, and normal controls, concluded that "evidence for a non–tau binding site and lack of correlation between tracer binding and antibody staining suggested that *in vivo* quantification of tau load with T-807/AV-1451 is problematic". This finding was preceded by the work of Vermeiren *et al* (6) reporting that AV-1451 has a high nanomolar binding affinity for MAO A and MAO B, enzymes abundantly present in the human brain in both neurons and glia which contributed to the 'off-target' *in vivo* AV-1451 PET signal. Displacement of [H-3]AV-1451 from its high affinity binding sites in human and rat brain homogenates with clorgyline, a selective MAO-A ligand, demonstrated unequivocally the 'off-target' ligand binding to the enzyme.

Whereas MAO A is widely distributed throughout the brain, MAO B has been shown to be predominantly present in the basal ganglia and related subcortical structures. The consequences of this non-specific, 'off-target' binding of AV-1451 are therefore very significant. The cortical brain localization of MAO A, in brain areas expected to have tau aggregates in various diseases, is a confounding factor and a severe limitation to any attempt to visualize and much less to quantify tau neuroaggregates with AV-1451 in the same regions. On the other hand, it was the likely MAO B-mediated non-specific label in the basal ganglia with AV-1451 that derailed the initial conclusions made on a single case of possible CTE in a football player, which was soon dismissed as erroneous (7). The work of Choi *et al* (in this issue of the Journal of Nuclear Medicine) provides additional important evidence that age-related 'off-target' AV-1451 binding in the basal ganglia is also closely correlated with iron accumulation as measured using iron sensitive R* magnetic resonance (MR) imaging.

Therefore, most of the problems associated with AV-1451 *in vivo* utilization do not seem entirely related to the strenuous requirements of tau/amyloid aggregate binding specificity for molecular imaging probes (8), but most importantly due to its non-specific binding to multiple tissue targets as indicated above. It may be surmised, however, that the possibility of T-807/AV-1451 binding to MAO enzymes could have been anticipated because it had already been known from the literature that 5H-pyrido[4,3-b]indoles were indeed potent MAO inhibitors (9). However, this

evidence was surprisingly unrecognized in the original publication (1), wherein it was reported that T-807/AV-1451 – a 5H-pyrido[4,3-b]indole - did not inhibit MAO A or MAO B.

It has also been a fundamental recognition that 'claims of AV-1451 high specificity for tau aggregates in AD were based primarily on autoradiography studies' which, as their design indicates, would fail to predict 'off-target' labeling. Undoubtedly, *in vitro* experiments are an important first step in PET probe development, but the limitations of these methods have been clearly established earlier in the literature (10). Still, *in vitro* experiments, no matter how carefully designed, are never capable of fully simulating the *in vivo* environment, for which *in vitro* to *in vivo* extrapolations should always be made with extreme care.

Cautionary voices have recently been raised to express concern for the disappointing observations with AV-1451, as well as other tau imaging agents (e.g., THK-5351), in a call for a humbling approach, '…to avoid making overarching claims that are supported by little data' (11). Also, as bluntly proclaimed, their lack of specificity may be 'a kiss of death' for several molecular candidates (11) and it'd be hard to disagree with the sagely expressed opinions of multiple investigators on the gloomy outcome of the so-called first generation of 'tau-specific' imaging agents. These results with AV-1451 (and other purported 'tau specific' imaging probes) should give us pause to fully comprehend the difficulties of PET probe development (10). Just injecting a radioactive compound with a limited and cursory validation to get a PET image is unfortunate with insightful PET biomarker development in order to get meaningful information.

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