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Tau positron emission tomography imaging in tauopathies: The added hurdle of off-target binding

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Abstract
Ligands targeting tau for use with positron emission tomography have rapidly been developed during the past several years, enabling the in vivo study of tau pathology in patients with Alzheimer's disease and related non-Alzheimer's disease tauopathies. Several candidate compounds have been developed, showing good in vitro characteristics with respect to their ability to bind tau deposits; off-target binding, however, has also been observed. In this short commentary, we briefly summarize the available in vivo and in vitro evidence pertaining to their off-target binding and discuss the different approaches that are needed for the future development of tau positron emission tomography tracers.
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1. Background

Recent advances in positron emission tomography (PET) have made possible the in vivo imaging of pathological forms of aggregated tau in Alzheimer's disease (AD) and related non-AD, or primary, tauopathies. The recognition of a key role for tau pathology in these neurodegenerative diseases, including an established correlation between neurofibrillary tangles, neuronal dysfunction, and clinical features [1], has accelerated the development of several families of tau PET ligands. Although several of these have shown favorable pharmacokinetic characteristics in vitro toward tau deposits and have also been included in AD clinical trials, the in vivo characterization of the tracers' binding in AD and non-AD tauopathies has been especially hampered by ongoing questions pertaining to tracer specificity and off-target binding.

The first published report of "off-target" binding in the context of in vivo tau imaging was based on findings showing that hippocampal retention of [18F]flortaucipir (formerly known as [18F]AV-1451, [18F]T807) in patients with mild cognitive impairment and AD did not increase with disease progression [2]. The authors speculated that this might be due to binding of the tracer to adjacent structures. Subsequent in vitro studies directed at this observation suggested that this putative binding of [18F]flortaucipir in the choroid plexus might be more "on-target" binding due to the identification of structures resembling Biondi "ring" tangles [3], as well as epithelial cells containing tau tangle-like structures and β-pleated sheet protein deposits [3]; in addition, electron microscopy evidence of paired helical filaments has been reported in this region [4]. Further postmortem studies, however, showed off-target binding of [18F]flortaucipir in neuromelanin-containing cells from the substantia nigra of progressive supranuclear palsy cases [5,6]. In this short commentary, we thus aim to briefly summarize the ongoing research involving tau tracers in different tauopathies, with a focus on highlighting the challenges inherent to their shared limitation of off-target binding.

2. In vivo tau PET and off-target binding

The three compound families that have thus far been most widely studied include [18F]flortaucipir, [11C]PBB3, and [18F]THK5317 and [18F]THK5351. Studies investigating their retention in vivo in clinically atypical parkinsonian syndromes associated with tau pathology (progressive supranuclear palsy and corticobasal degeneration) reported binding primarily in the basal ganglia and, secondarily, in distinct cortical areas, consistent with the neuropathological literature [7]. To date, however, studies investigating the potential discrimination in terms of retention between these syndromes and age-matched healthy volunteers have produced equivocal results [7,8]. The aforementioned inconsistency probably derives from the off-target signal of those tracers in the basal ganglia [7]; although basal ganglia structures are relatively spared of tau burden in syndromes not related to parkinsonism, tracer signal of moderate-to-high intensity is detected in healthy volunteers and patients with AD (Fig. 1). Furthermore, the off-target signal in the basal ganglia is reported consistently, although with different intensity, across all tau tracers [7,9]; although binding to monoamine oxidase B (MAO-B) presumably explains most off-target binding in this region, the exact origins have yet to be firmly established.

While preliminary evidence has shown similar off-target binding in head-to-head studies [9,10], multitracer antemortem/postmortem designs incorporating these comparisons, as well as blocking experiments, are crucial to fully characterize the binding properties of these ligands.

The only antemortem/postmortem tau PET study published so far showed binding of [18F]THK5351 to MAO-B in AD, a finding consistent with the off-target signal of tau tracers in the MAO-B-rich basal ganglia [11]. In a related in vivo study, administration of a MAO-B inhibitor led to a global decrease in [18F]THK5351 signal, quantified using standard uptake values. When using a standard reference region–based approach (standard uptake value ratios), however, the authors reported no statistically significant decreases in [18F]THK5351 retention [12]. While the lack of significance when using standard uptake value ratios likely reflects a decline in MAO-B availability in the reference region as a result of the pharmacological challenge, an alternative explanation may involve altered brain perfusion, and thus delivery of the tracer, possibly via changes in blood pressure [13]. While a recent retrospective study involving Parkinson's disease patients treated chronically with MAO-B inhibitors showed no effect on [18F]flortaucipir uptake [14], due to
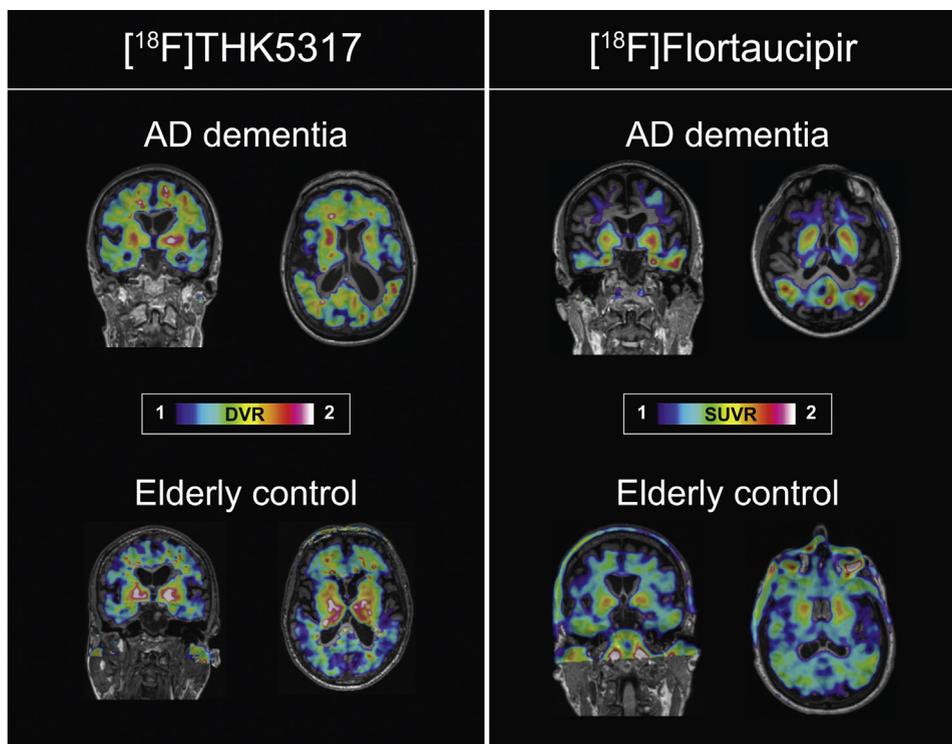
Fig. 1. Representative [18F]THK5317 DVR (40–60 minutes) and [18F]flortaucipir ([18F]AV-1451) SUVR images (75–105 minutes) from AD dementia patients (top row) and elderly controls (bottom row). [18F]Flortaucipir images were obtained from the ADNI database (http://adni.loni.ucla.edu/). Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; DVR, distribution volume ratio; SUVR, standard uptake value ratio.

the between-study differences, it cannot be ruled out that the pretreatment could affect the availability of the binding site for [18F]THK5351.

Vascular structures (i.e., choroid plexus and dural venous sinuses) represent another off-target binding area for two of the developed tau tracers ([18F]flortaucipir and [11C]PBB3) [7]. The localization of the choroid plexus in the lateral ventricles, in close proximity to the hippocampus and of the dural venous sinuses in the cerebellum, could lead to problematic quantification of the tracer retention, especially with the use of conventional low-resolution PET systems and without the application of partial volume correction. While the hippocampus might not appear of interest in non-AD tauopathies, the cerebellum represents the only reference region used for the quantification of all tracers, and potential spillover from the dural venous sinuses into the cerebellum could lead to substantial underestimation of the tracer retention in regions of interest. Additional binding to α-synuclein has been reported for [11C]PBB3 [15]. Further, binding to transactive response DNA binding protein 43 was speculated to explain the in vivo uptake patterns of [18F]flortaucipir [16] and [18F]THK5351 [17] in a small number of patients with the semantic variant of primary progressive aphasia, based on the resemblance of the in vivo uptake pattern to the known distribution of transactive response DNA binding protein 43 pathology; postmortem validation of these findings, however, is still pending. So far, all tracers have shown in vitro specificity to tau over amyloid β deposits [7], although in vivo off-target binding to the latter β-sheet structure cannot be ruled out at present.

3. Understanding the complexity of tau PET binding: what can be learned from in vitro studies?

In vitro binding studies performed so far using both immunohistochemistry and autofluorescence have shown a complex pattern of binding for tau tracers, including specific binding to neurofibrillary tangles [5,18,19], but also off-target binding in the choroid plexus, to melanin and lipofusein structures [5], mineralized structures [5], MAO-A and MAO-B [20,21], tufted astrocytes [22], white matter [23], and dense core amyloid plaques [24]. Furthermore, differences in binding properties have been observed across the different tau isoforms present in AD and non-AD tauopathies [7]. However, most of the in vitro work for non-AD tauopathies has been performed on paraffin sections with heating and fixing steps possibly modifying the different binding sites. We can cite, for example, [3H]deprenyl specifically targeting the MAO-B enzyme, for which autoradiography on paraffin sections cannot be performed, most probably due to damage to the enzyme during the paraffin embedding process. In our in vitro studies, we demonstrated that while [3H]deprenyl and [3H]THK5117 showed a similar cortical laminar distribution, they were not competing at the concentration range used in PET studies (4 nM), indicating that they do not bind to the same site, or at least not with the same affinity [25]. Moreover, while the highest MAO-B inhibition of [18F]THK5351 binding reported by Ng et al. [26] was 50% in the striatum at 500 nM, we observed only 25% inhibition at 150 nM, a concentration level well beyond that used in PET. Almost all tau PET tracers from the first generation seem to show
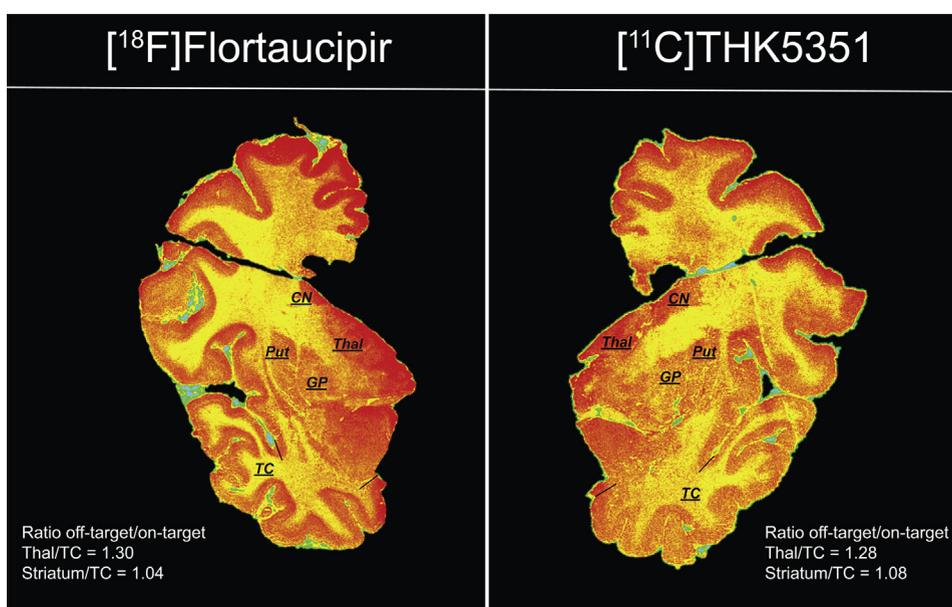
Fig. 2. Autoradiography showing total binding of [18F]flortaucipir (0.04 nM) and [11C]THK5351 (0.4 nM) on the large coronal frozen section from an AD case. Abbreviations: CN, caudate nucleus; GP, globus pallidus; Thal, thalamus; Put, putamen; TC, temporal cortex; Striatum: CN + Put. For methodological details, see [28].

MAO-B off-target binding, however, which could mean something about the comparability of tau and MAO-B as targets.

Although [^{18}F]THK5351 is, to our knowledge, the only tau PET tracer where the MAO-B component has been tested in vivo via pretreatment with selegiline [12], [^{3}H] flortaucipir has shown similar in vitro affinity toward MAO-A and MAO-B [27], and unlabeled flortaucipir and THK5117 have shown a similar level of affinity toward MAO-B [28]. Indeed, using in vitro autoradiography, a similar regional distribution and "off-target/on-target" binding ratio were observed for [^{18}F]flortaucipir and [^{11}C]THK5351 (Fig. 2). In vivo studies incorporating pretreatment with MAO-B inhibitors before tau PET imaging may help clarify the degree to which tau PET tracers bind to MAO-B. Finally, several novel candidate tau tracers have recently been developed, and in vitro characterization has been described both in the literature and at international conferences [29]. These tracers appear to have lower off-target binding compared to what has been reported thus far for the first generation of tau ligands; more in vivo studies, however, are needed to confirm their superior performance [29].

4. Conclusion

The development of tau PET tracers has occurred rapidly over the course of the past several years. Currently available tau PET tracers seem to be able to bind pathological tau in vivo, including, it is assumed, intracellular forms; while several imaging studies have indeed shown a close correspondence between Braak staging of tau pathology and ligand retention in AD [30–32], as well as expected ligand retention patterns in non-AD tauopathies, such as progressive supranuclear palsy [33], off-target binding remains an outstanding limitation. Further studies addressing how this issue impacts signal quantification are thus of importance both to allow for continued use of first-generation tracers and to ensure that this shortcoming does not remain a problem for second generation tau ligands. In this respect, the availability of the cryo-electron microscopy structure of AD tau filaments [34] may serve as a helpful starting point for future in silico studies aiming, among other things, to establish the exact proportion of binding sites occupied by each tracer and how differences in the proportion of occupied sites may affect the PET signal seen in vivo. In the future, combinatorial approaches involving in silico modeling with in vitro characterization and in vivo comparison will be crucial to better understand the binding properties of tau PET tracers and to clearly identify what they are binding to.

Acknowledgments

RESEARCH IN CONTEXT

1. Systematic review: We reviewed the existing in vivo and in vitro literature related to tau positron emission tomography (PET) imaging in PubMed, as well as abstracts from recent international conferences and our own experimental data, with a focus on off-target binding.

2. Interpretation: We briefly summarized the available data for off-target binding with the first-generation tau PET tracers, including [^{18}F]flortaucipir and the [^{18}F]THK family of compounds. Similarities in off-target binding were observed between [^{18}F]flortaucipir and [^{18}F]THK compounds.

3. Future directions: The substantial presence of off-target binding across first-generation tau tracers hinders the clinical applicability of tau PET imaging, especially in primary tauopathies. We suggest that greater efforts need to be directed toward approaches integrating in silico, in vitro, and in vivo techniques in the design of next-generation tau tracers to achieve the lowest possible off-target binding, thus allowing tau PET to enter into clinical applications in the near future.

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