The Merits of FDDNP-PET Imaging in Alzheimer’s Disease

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Abstract. 2-(1-[(6-[(2-[fluorine-18]fluoroethyl)(methyl)amino]-2-naphthyl)ethylidene]malononitrile (FDDNP) is the first positron emission tomography (PET) molecular imaging probe to visualize Alzheimer’s disease (AD) pathology in living humans. The most unique features of FDDNP are that (1) it is the only currently available radiotracer to image neurofibrillary tangles, beside amyloid aggregates, in living humans; and (2) it is also the only radiotracer to visualize AD pathology in the hippocampal region of living humans. In this article, we discuss FDDNP’s unique ability to image tau pathology in living humans. Emphasizing tau pathology imaging capability using FDDNP in AD, as well as other tauopathies, is timely and beneficial considering that (1) post mortem histopathological studies using human specimens have consistently demonstrated that neurofibrillary tangles, compared with amyloid plaques, are better correlated with the disease severity and neuronal death; and (2) recently reported clinical trial failures of disease-modifying drugs in development, based on the amyloid-cascade hypothesis, suggest that some of the basic assumptions of AD causality warrant reassessment and redirection.

INTRODUCTION

The first attempt to visualize Alzheimer’s disease (AD) pathology in the brain of living humans using positron emission tomography (PET) was presented in preliminary form by Barrio and associates in 1999 [1], and the first full report of this work appeared in 2002 [2]. This radiotracer is 2-(1-[(6-[(F-18)fluoroethyl](methyl)amino)-2-naphthyl]ethylidene)malononitrile (FDDNP)[3], which was demonstrated to be effective in the measurement of in vivo brain cortical accumulation of both amyloid plaques and neurofibrillary tangles in living subjects using PET [2, 4–8]. The first carbon-11-labeled PET radiotracer intended for human in vivo amyloid-beta imaging known as Pittsburgh Compound-B (PIB) was presented in preliminary form in 2002, with the full report in 2004 (see [9] for review)1.

After FDDNP and PIB appeared, results from human studies with at least five other amyloid-beta imaging PET probes have been reported (11C-SB-13 [12]; 11C-BF-227 [13], 18F-BAY94-9172 (Florbetaben) [14], 18F-AV-45 (Florbetapir) [15], and 18F-Flutemetamol [16]). The most unique feature of FDDNP is its ability for structural recognition of β-sheet aggregate conformations in neuroaggregates (e.g., β-amyloid, tau and prions) – like Congo Red and Thioflavin-T [17]. Moreover, it is the only imaging probe to allow the visualization of neurofibrillary tangles as well as amyloid plaques in living humans. In

1 Note that there are new data on the in vivo binding properties of PIB (see [10] and [11]).
addition, FDDNP is the only imaging probe which successfully visualizes AD pathology in the hippocampal region of living humans.

In this article, we discuss FDDNP’s merits based on its unique ability to image tau pathology in living humans. While β-amyloid plaques and neurofibrillary tangles are two representative hallmarks of AD pathology, the amyloid cascade hypothesis, a widely proposed pathogenic mechanism for AD [18], has shifted the attention to β-amyloid aggregates in the assumption that tangle formation is a direct consequence of amyloid plaque formation. However, several lines of histopathological evidence demonstrate that neurofibrillary tangles antecedent β-amyloid plaques in AD [19–23]. Moreover, post mortem histopathological determinations using human brain specimens have consistently demonstrated that neurofibrillary tangles, and not β-amyloid plaques, are best correlated with the severity of cognitive impairment (e.g., mini-mental state examination, MMSE) in normal aging, mild cognitive impairment (MCI), and patients with AD [24–27]. Finally, recently repeated clinical trial failures with disease-modifying drugs based on the amyloid cascade hypothesis cast significant doubts on the basic assumptions of AD causality [28]. Therefore, the potential benefits of tangle pathology imaging using FDDNP in AD and other tauopathies cannot be underestimated.

FDDNP VISUALIZES NEUROFIBRILLARY TANGLES besides β-AMYLOID PLAQUES

Earlier in vitro studies initially suggested FDDNP binding to β-amyloid plaques and tangles and its potential for in vivo use [3]. Moreover, binding of FDDNP to brain β-amyloid aggregates in transgenic rat models (e.g., in a homozygous triple-transgenic rat model of AD [Tg470/Tg1116/Tg11587]) present excellent correlations (ELISA) with amyloid content [29]. Also, aged rhesus monkeys develop amyloid plaques without neurofibrillary tangles in the brain [30]. A recent study with FDDNP-PET imaging in aged adult male rhesus monkeys reported that brain regions known to have amyloid plaque deposition showed increased FDDNP uptake [31], yet no post mortem determination of β-amyloid load was performed in this study. These results support the conclusion that FDDNP binds to amyloid plaques in the brain in vivo. Similarly, several lines of evidence support FDDNP binding to tau aggregates. First, the DDNP moiety co-crystalizes with tau segments (VQIVYK) and the structure of the crystal was resolved at atomic resolution [32]; FDDNP also labels cell transfected with tau constructs (SY5Y) and also labels tau aggregates in MAPT transgenic mice (G. Cole et al, personal communication). Also, in humans FDDNP accumulation in hippocampus has been correlated with abundant tau tangle accumulation in this area [5], consistent with FDDNP labeling of tau-containing protein aggregates in brain specimens of various neurodegenerative diseases [4, 17]. In living patients FDDNP-PET succeeded in showing a pattern of neurofibrillary tangle distribution in patients with frontotemporal dementia [33], a disease with abundant brain neurofibrillary tangles without significant β-amyloid plaque deposition in the brain [34]. Similarly, in progressive supranuclear palsy (PSP) – a neurodegenerative disease lacking amyloid and with various tau-positive abnormal aggregates. FDDNP-PET shows imaging patterns highly consistent with the presence of tau aggregation in subcortical structures (e.g., brain stem, caudate, putamen, thalamus) and cerebellum extending towards cortical structures with disease progression [35]. Moreover, a recent report of a positive association between FDDNP and CSF tau in AD [36] further supports the binding of FDDNP to tau.

Finally, FDDNP-PET results performed independently at three different sites agreed with the observation that medial temporal cortical FDDNP binding is consistently higher than neocortical FDDNP binding in AD [2, 5–7]. Previous post mortem histological studies demonstrated that, in AD, β-amyloid-plaque deposition is high in neocortical regions but relatively lower in some medial temporal cortical substructures, e.g., hippocampus [37], even though it is high in entorhinal cortex [10, 38]. By contrast, tangle accumulation is high in the medial temporal cortex at all levels of cognitive impairment with subsequent neocortical increases with disease progression [37, 39]. Taken together, the available data on FDDNP in vivo binding patterns and neuropathology indicate that FDDNP is a sensitive in vivo marker for neurofibrillary tangles.

FDDNP BINDING MATCHES THE PATTERN OF BRAIN PATHOLOGY AND CLINICAL PROGRESSION IN AD

As noted above, post mortem histopathological determinations with human brain specimens have
Fig. 1. Panel (A) shows a hemispheric surface map of FDDNP DVR progression/spread as mini-mental state examination (MMSE) scores decrease. We see a similar trend between the left side and the right side. There is a bit of signal in the left temporal lobe at a normal MMSE of 30 that increases in the temporal lobe and spreads to the parietal and frontal areas as MMSE drops. This DVR spreading mimics the pathology progression of beta-amyloid plaques and neurofibrillary tangles accumulation in Alzheimer’s disease. Panel (B) shows the surface map of regression slope of FDDNP DVR increase per unit change of MMSE score. The higher the slope corresponds to a faster increase of the FDDNP DVR. The areas of highest slope match areas of significant beta-amyloid plaques and neurofibrillary tangles in AD that include the lateral temporal, lateral frontal, anterior cingulate, medial temporal and posterior cingulate areas. Panel (C) shows a cortical surface map of F-statistics of the linear regression model between FDDNP DVR and MMSE. Areas that have a significant slope with $p = 0.05$ are shown with colors above dark blue. It is important to note that lateral frontal, lateral temporal, medial temporal, anterior cingulate and posterior cingulate all have a significant slope, and all have been shown to be important in AD. Adapted and reprinted with permission from Protas et al. (2010) [Copyright (2010) Neuroimage].

Consistently demonstrated that neurofibrillary tangles, compared with beta-amyloid plaques, are better correlated with the severity of cognitive impairment (e.g., mini-mental state examination [MMSE] or clinical dementia rating [CDR] in normal aging, MCI, and patients with AD) [23–27, 40–44]. Consistent with these post-mortem studies, FDDNP-PET imaging studies performed in normal aging, MCI, and patients with AD show significant correlations with MMSE scores [45, 46], while PET imaging studies using radiotracers (e.g., PIB and BF-227) thought to bind amyloid plaques without binding to tangles do not [47, 48]. In addition, FDDNP signal progression in AD matches the progressive accumulation of brain pathology described by Braak and Braak [37] (see Fig. 1). By contrast, PIB has not shown a progressive pattern as expected from autopsy data [37]. For example, MCI patients show an “on and off” PIB binding pattern that is either negative, that is similar to controls (which is difficult to explain since brain pathology deposition is generally present in MCI) or positive (i.e., similar to AD) [49–51]. Further, PIB binding in the precuneus has been found to be the highest of all cortical areas [52], yet autopsy studies have not found higher beta-amyloid deposition in the precuneus compared with other cortical areas [10].

**FDDNP-PET SHOWS SUPERIOR PERFORMANCE COMPARED WITH FDG-PET AND MRI**

Imaging techniques, such as 2-deoxy-2-[18F]fluoro-D-glucose (FDG)-PET and structural magnetic
resonance imaging (MRI) have been investigated to classify normal aging, MCI, and patients with AD. Small et al. [5] found global FDDNP binding values to be more accurate than previously established sensitive measures for FDG-PET [53–55] or volumetric MRI measures [56–58] for diagnostic classification of subjects.

In these comparisons, values for FDDNP-PET global binding were more effective in discriminating among diagnostic groups than FDG-PET glucose metabolism in the posterior cingulate gyrus or parietal regions [53, 54] or MRI volumes of the medial temporal regions, which many clinicians currently rely upon for diagnostic confirmation of AD [57].

**FDDNP BINDING IS RELATED TO NEURONAL LOSS IN THE HIPPOCAMPUS**

Neurofibrillary tangle density correlates more strongly with neuronal death than does total plaque burden [59]. Large pyramidal neurons in the CA1 and subicular regions of the hippocampus, a part of the medial temporal lobe system supporting declarative memory, are among the most vulnerable neuronal populations affected in the earliest stages of the disease in a pattern that differs from that of normal aging, and is highest in entorhinal cortex and hippocampus CA1 region [60]. Although these large pyramidal neurons are glutamatergic in nature, they receive inhibitory serotonergic input from the dorsal raphe nucleus via the serotonin 1A (5-HT1A) receptors located on their axonal hillock. Hippocampal CA1 region and subiculum have the highest density of 5-HT1A receptors in the brain, therefore, a decrease in 5-HT1A receptor densities in the same areas in AD can be demonstrated in vivo by using 4-[F-18]fluoro-N-[2-[1-(2-methoxyphenyl)piperazinyl]ethyl]-N-([2-pyridinyl]benzamide (MPPF) PET in patients and correlated with the hippocampal cellular pathology, thus using them as surrogate marker of hippocampal degeneration in AD, including pyramidal neuron loss in hippocampus [61].

To investigate the relationship between FDDNP binding and hippocampal degeneration, FDDNP-PET and MPPF-PET scans were performed, and hippocampal MPPF binding values were found to closely correlate with cortical increases in FDDNP binding. Moreover, they were also strongly correlated with the severity of cognitive decline in AD, as measured by MMSE scores [45, 61]. Particularly remarkable is that hippocampal 5-HT1A densities were found to be decreased in MCI subjects, making their assessment a possible target for early diagnosis of AD [61]. Densities of hippocampal 5-HT1A receptors at pre-clinical stages of AD are affected by two opposing processes: pyramidal neuron losses in CA1 and subiculum regions causing decreases in regional receptor density, but these decreases are counteracted by increased receptor expression on surviving pyramidal neurons. In contrast to our observations, Truchot and colleagues [62] initially reported increases in hippocampal 5-HT1A receptor density in their amnestic MCI population compared to controls measured by MPPF-PET and attributed it to compensatory mechanisms. In their subsequent paper [63] on measurement of 5-HT1A receptor densities with MPPF-PET, they observed decreased hippocampal 5-HT1A receptor densities in the amnestic MCI group compared to controls, similar to our results [61], and increased receptor density in several neocortical regions. When the disease reaches clinical stages, hippocampal pyramidal neuronal losses and volume loss in the CA1 region of the hippocampus and subiculum [64, 65] prevail over compensatory effects, and significantly decreased hippocampal 5-HT1A receptor densities have been observed in all studies [61–63]. The combined evaluation of FDDNP-PET (targeting tangles and plaques) with MPPF-PET (targeting serotonin 1A receptors as a marker of hippocampal degeneration) offers the opportunity for reliable, non-invasive detection of both AD pathology and hippocampal neuronal loss.

By contrast, the relationship between PIB binding and hippocampal neuronal loss is controversial: While some studies found that global and regional atrophy were strongly related to PIB binding in subjects with subjective cognitive impairment, no such relationship has been found in patients with MCI or AD [66]. Other reports have found no associations detected between current PIB binding and regional brain volume decline trajectories in preceding years [67].

**MULTITRACER PET IMAGING USING BOTH FDDNP AND PIB**

Because of their different imaging characteristics, multitracer PET imaging using PIB and FDDNP in the same subjects may help visualize different aspects of AD development. To investigate this issue,
Patients with AD have consistently shown low PIB but high FDDNP binding in the medial temporal cortex (limbic regions including hippocampus, parahippocampal areas, and entorhinal cortex), while both PIB and FDDNP binding are significantly increased in neocortical areas in AD compared with age matched controls (See Fig. 2). Consistent with these results, subtracted PET data (FDDNP minus PIB) acquired from the same patients with AD (analyzed using statistical parameter mapping software [8]) found that the medial temporal cortex was the most significant differential brain region in the voxel mapping (see Fig. 3).

Post mortem studies describing the hierarchical progression of tau lesions in normal aging and early stages of AD suggest that damage to the medial temporal cortex and association cortex would account for the memory and non-memory cognitive impairments, respectively [68]. Therefore, high FDDNP binding observed in the medial temporal cortex of patients with AD is consistent with the suggested relationship between the medial temporal tau pathology and memory impairment in AD.

**MEDIAL TEMPORAL FDDNP BINDING IS RELATED TO EPISODIC MEMORY PERFORMANCE**

Episodic memory impairment is one of the most prevalent cognitive deficits in patients with AD [69], and a subtle decline in episodic memory often occurs prior to the emergence of the full dementia syndrome in non-demented older adults who eventually develop AD [70].

The medial temporal cortex has been well-established as playing a central role in modulating episodic memory [71]. We found that medial temporal FDDNP uptake values in normal elderly subjects are correlated inversely with long delay recall scores in the California Verbal Learning Test ($r = -0.684$, $p = 0.029$), an instrument used to test episodic memory performance [6]. By contrast, medial temporal PIB binding does not show a statistically significant correlation with episodic memory performance [6]. These findings may suggest that episodic memory impairment might be related to the presence of intraneuronal neurofibrillary tangles in the medial temporal cortex.
Post mortem pathological evidence previously showed that neurofibrillary tangle density in the medial temporal cortex correlates with memory scores, whereas density of amyloid plaques in the same region does not [72, 73]. Determining the details of the connections between tau pathology and episodic memory impairment warrants further study, but it is expected that tangle formation may at least partly contribute to episodic memory deterioration via impairing electroencephalogram (EEG) theta rhythm [74–76].

**FDDNP BINDING PATTERN IN THE BRAIN’S DEFAULT NETWORK**

The concept of a default mode network – an interconnected set of brain regions (frontal, parietal, posterior cingulate, lateral, and medial temporal regions) that is active when the brain is in a resting state and deactivated during focused mental tasks -- was first proposed in 2001 [77]. This concept rapidly became the target of hot research in AD, where such a default network appears disrupted, most prominently in the medial temporal cortex [78–80]. FDDNP binding patterns in patients with AD show significantly increased binding values in the entire default network, including the medial temporal cortex [5, 6], which points to the utility of FDDNP-PET in identifying the role of the default network in AD [81].

**QUANTITATIVE ANALYSIS OF FDDNP-PET USING SUBCORTICAL WHITE MATTER AS REFERENCE REGION**

There is imprecise information in the literature regarding non-specific white matter binding of FDDNP and other probes. Recently, Wong et al. [82] investigated this issue and found that for FDDNP, the ratio of white matter over cerebellum is 1.0, whereas for PIB the white matter/cerebellum ratio is 1.5; that ratio is even higher for imaging probes structurally related to trans-stilbenes (e.g., AV-45). Therefore, the cortex/cerebellum ratio for expressing a signal is misleading and the cortex/white matter ratio would better express the signal to noise ratio in adjacent tissues. High white matter signal produces a significant spill-over effect of the signal to the cortical grey matter. It also adds to partial volume effects, which distort results, most particularly in AD when significant cortical atrophy is present. These effects can be compensated to some extent by analytical approaches that use segmentation of grey and white matter based on MRI scans before quantitation of PET data to determine grey matter cortical binding of these tracers, yet the question of the origin of high white matter binding of these tracers still remains.

Because the white matter/cerebellum ratio for FDDNP uptake is 1.0 [82], subcortical white matter is a good alternative reference region to the cerebellum for analyzing FDDNP-PET data, particularly when the cerebellar region is affected by disease (e.g., in prion disease) [83]. Wong and colleagues [82] have shown that lower perfusion of white matter compared to grey matter does not present a problem for Logan graphical analysis for this tracer and that it does not increase inter-subject variability over that observed in analysis performed with the cerebellum as the reference region. In practice, both the subcortical white matter and the cerebellum should be used together to cross-validate the findings with different reference regions, especially when arterial input blood data are not available or blood sampling is not feasible due to practical clinical considerations. Comparison of both approaches was tested in 10 AD and 10 control subjects and results show very good correlations [83, Supplementary Materials].

**THE LIMITATIONS OF FDDNP-PET**

One of the most frequently mentioned limitations of FDDNP is the small cortical signal difference between control and AD subjects [9]. The in vivo FDDNP binding differences between controls and AD patients are significantly lower (approx 10-15%) than those of PIB-PET (approx 80%) in some neocortical regions when the cerebellum is used as a reference [6]. That difference is reduced to about 25-30% for PIB-PET (vs 10-15% for FDDNP-PET) when the adjacent white matter region is used as a reference [82]. It also should be noted that in the medial temporal region, the cortical area with the earliest pathology deposition in AD, the FDDNP binding difference between controls and AD is much higher (approx 30 %) compared with PIB-PET (approx 5%) which shows the lowest level of signal of all brain regions [6]. To demonstrate this characteristic between FDDNP-PET and PIB-PET, we show the differential PET image (FDDNP minus PIB) in Fig. 3, where PIB binding values are shown to be higher than FDDNP binding values in several neocortical regions and FDDNP binding values are higher than PIB binding values in the medial temporal cortical regions [8].
Unlike PIB-PET, which shows a single pattern of affected areas at all stages of AD progression, we have observed an increasing pattern of FDDNP cortical brain involvement with disease progression consistent with known pathological data at all levels of neurological impairment [46]. This may increase the difficulty in quantification of FDDNP-PET data if group separation (e.g., controls vs. AD) is intended, yet it more closely matches the stepwise evolution of pathology pattern in AD (see Fig. 1).

Another often mentioned critique of FDDNP is that it does not bind to β-amyloid plaques selectively. However, this argument is strongly biased by the influence of the amyloid cascade hypothesis which considers β-amyloid as causative of AD, in contrast to other criteria which attributes the disease to other factors (e.g., inflammation) and considers both neurofibrillary tangles and β-amyloid plaques as pathological diagnostic hallmarks of AD. In AD both pathologies are contributing to disease and thus binding to both Alzheimer’s pathologies could be considered an advantage since evolution of these pathologies follows different time- and space-related patterns. Recent repeated failures in developing anti-amyloid drugs and vaccines cast doubts on the amyloid cascade hypothesis for which the ability of FDDNP to image both plaque and tangle would be considered favorably rather than an obstacle in AD diagnosis and drug treatment monitoring, particularly at the earliest stages involving the medial temporal lobe where PIB-PET shows no signal [84]. Therefore, even with the relatively low neocortical signal, FDDNP value as an imaging agent is enhanced by two important facts: (1) at present, it is the only clinically available in vivo imaging probe to visualize tau pathology in living humans; and (2) it is also the only radiotracer to visualize AD pathology in the hippocampal region of living humans, which is among the earliest affected brain region in AD.

THE POTENTIAL ADVANTAGE OF FDDNP-PET IN THE ASSESSMENT OF EARLY ALZHEIMER’S DISEASE AND/OR THOSE SUBJECTS AT RISK FOR AD

The amyloid cascade hypothesis assumes that amyloid plaque formation precedes neurofibrillary tangle formation [18]. Supporting evidence for the amyloid cascade hypothesis comes from early-onset familial AD cases caused by mutations in three different genes, amyloid precursor protein (APP) and presenilin-1 and -2 (PS1 and PS2). However, less than 5 percent of all Alzheimer cases are early-onset familial AD. By contrast, human postmortem histopathology data suggest that the initial development of tangles might precede the development of amyloid plaques by at least two decades [20, 21]. The progression of tangle formation is stepwise and consistent from the entorhinal cortex, through the hippocampus, and into the isocortex [37]. Therefore, early detection of AD pathology in living humans at its onset before it spreads widely will contribute to the early, preclinical diagnosis of, and early treatments for, AD. If so, because of its sensitivity for tau aggregates, FDDNP-PET imaging would have an advantage in an early, preclinical diagnosis of AD, especially in imaging medial temporal cortex.

CONCLUSIONS

Previously well-established post mortem human data suggest that tau tangles are more accurate predictors than amyloid plaques in monitoring disease stages and cognitive performance in normal aging, MCI, and AD. Consistent with post mortem studies, FDDNP-PET imaging scans performed in normal aging, MCI, and patients with AD show significant correlations with MMSE scores, as well as MPPF-PET hippocampal neuronal loss measures, while other PET imaging probes purportedly designed to bind amyloid pathology do not. In addition, FDDNP signal progression in AD also matches the progressive accumulation of brain pathology described by post mortem histopathological studies. Furthermore, FDDNP is the only imaging probe to succeed in visualizing AD pathology in the medial temporal cortex (especially, in the hippocampus) of living humans, and thus the FDDNP binding in the medial temporal cortex is expected to associate with predominant tangle formation in the region, episodic memory impairment, the brain’s default network, and early, preclinical diagnosis of AD. Therefore, FDDNP-PET, which can visualize neurofibrillary tangles in addition to amyloid plaques, provides unique information on AD brain pathology relevant to disease progression.

FINANCIAL DISCLOSURE

The University of California, Los Angeles, owns a U.S. patent (6,274,119) entitled “Methods for Labeling β-Amyloid Plaques and Neurofibrillary
Tangles,” that uses the approach outlined in this article. Drs. Small and Barrio are among the inventors, have received royalties, and may receive royalties on future sales. Dr. Small reports serving as a consultant and having received lecture fees from Nihon Medi-Physics Co, Bristol-Meyer Squibb, PETNet Pharmaceuticals, and Siemens.

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