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Short Research Article

Radiotracers for a multi-target approach to the diagnosis of Alzheimer's disease†

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Abstract: Development of β-amyloid plaques and neurofibrillary tangles is the hallmark of Alzheimer's disease (AD) and progressively affects the overall functioning of the brain. Noninvasive imaging methods aiding early diagnosis will significantly improve benefits provided by treatments and possibly lead to prevention of AD. We report the development of PET radiotracer methods for β-amyloid plaques and tangles, nicotinic $\alpha 4\beta 2$ receptors, serotonin 5HT1A receptors, dopamine D2/D3 receptors and norepinephrine transporters for the study of AD. This multi-target approach is currently being evaluated in AD transgenic mice models.

Keywords: PET; ^{18}F-FBM; ^{18}F-Nifene; ^{18}F-Mefway; ^{18}F-Fallypride; ^{18}F-MFP3

Introduction

Alzheimer's disease (AD) is characterized by the accumulation of two characteristic pathological features in the brain, β-amyloid plaques and neurofibrillary tangles. Diagnosis and patient follow-up are by a battery of cognitive and behavioral tests. This has allowed classification of patients as having possible or probable AD based on the extent of cognitive impairment of daily living functions. However, a definitive diagnosis of AD cannot be made until a postmortem examination of the brain. Noninvasive diagnostic imaging modalities such as positron emission tomography (PET) may aid in the diagnosis of AD. Changes in glucose metabolism using ^{18}F-fluoro-2-deoxyglucose (^{18}F-FDG) in AD have been studied for at least 10 years. Characteristic features of AD include lower glucose metabolism measured with ^{18}F-FDG in the parietotemporal cortex, cingulate and other brain regions that correlate with disease severity.[1] Although ^{18}F-FDG has served well in the study of AD, and is currently being explored under an AD Neuroimaging Initiative trial at several sites, a need for more sensitive radiotracers (or biomarkers) for the early and accurate diagnosis of AD is needed.

Since AD involves the development of β-amyloid plaques and neurofibrillary tangles, over the last 5 years progress has been made on PET radiotracers that are able to image these processes.[2] Studies using these imaging agents are shedding new light on the association of plaques and tangles with the progression of the disease. Functional consequences of β-amyloid plaques and tangle accumulation include disruption of several CNS processes affecting neurotransmission. One of the early radiotracer studies in AD population was to evaluate acetylcholinesterase, since cholinergic deficits in AD subjects have been recognized early on.[3] Acetylcholinesterase inhibitors, whose putative mode of action is to increase acetylcholine levels, are currently used to treat AD with some success.[4]

Progressive β-amyloid plaque and neurofibrillary tangle accumulation is thought to lead to a gradual loss of neuronal function. Several neurotransmitter receptor systems have been implicated in this process

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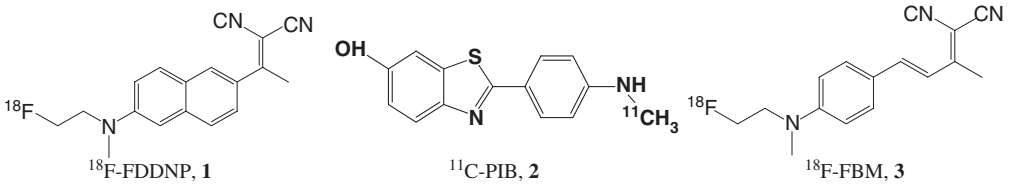
of degeneration. We are therefore developing a multitarget approach to detect AD that will encompass the following systems: (1) plaque and tangle imaging; (2) nicotine $\alpha 4\beta 2$ receptors; (3) serotonin 5HT1A receptors; (4) extrastriatal dopamine receptors; (5) norepinephrine transporters (NET); and (6) neurotransmitter measurements. These radiotracer methods will allow: (a) evaluation of plaques and tangles in transgenic animal models (preclinical studies) of AD as well as in patients with AD (both postmortem brains and living brain); (b) evaluation and correlation of deficits in neurotransmitter-receptor functions with plaque and tangle formation in transgenic animal models of AD as well as human AD (both postmortem brains and living brain); (c) two animal models are being used in our laboratory: (1) Tg2576 mouse (develops Aβ plaque pathology); and (2) 3xTg-AD mouse (develops both Aβ plaques and neurofibrillary tangles). These methods will ultimately be useful in early diagnosis, treatment planning and the development of new therapeutics for AD.

Results and discussion

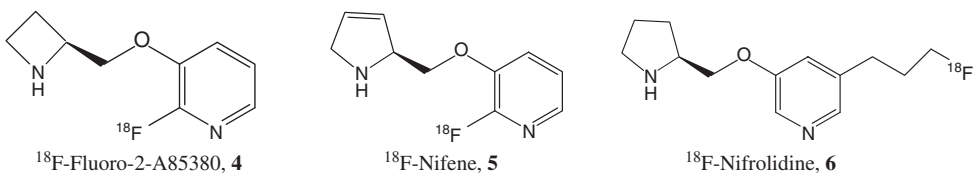
1. *Plaque and tangle imaging*: Imaging of plaques and tangles has been currently underway with various classes of compounds. Two agents in particular that are being evaluated in human studies include ^{18}F-FDDNP (Figure 1, **1**, for plaques and tangles) and ^{11}C-PIB (Figure 1, **2**, for plaques). The dinitrile derivative, ^{18}F-FDDNP, binds to β-amyloid plaques and neurofibrillary tangles but is considered to be lipophilic ($\log P > 3$) and thus provides lower target-to-nontarget ratios.[5] The other agent is ^{11}C-PIB, which is suitable for imaging β-amyloid plaques.[6] Our objective has been to image both, β-amyloid plaques and tangles in mouse transgenic models as well as in humans. We have thus designed a less lipophilic analog, ^{18}F-FBM (Figure 1, **3**, for plaques and tangles), in order to facilitate the study of these processes.[7] Radiosynthesis of ^{18}F-FBM was carried out by an exchange of the corresponding tosylate with nucleophilic fluorine-18 as described for ^{18}F-FDDNP. Preliminary studies with ^{18}F-FBM in triple transgenic AD mice that develop both β-amyloid plaques and tangles[8] is consistent with binding to β-amyloid plaques identified using immunolabeling with an anti-Aβ42 antibody and thioflavin S staining. Nonspecific binding with ^{18}F-FBM was found to be lower compared to ^{18}F-FDDNP, thus providing better ratios between areas containing the β-amyloid plaques (hippocampus) versus nonspecific binding in the cerebellum.

2. *Nicotinic receptor imaging*: The nicotinic acetylcholinergic system has been shown to be involved in AD and implicated in cognitive decline in this patient population. Specifically, loss of $\alpha 4\beta 2$ receptors has been reported in postmortem studies in AD.[9a] Using ^{18}F-fluoro-2-A85380 (Figure 2, **4**) in PET studies of AD patients confirms loss of the $\alpha 4\beta 2$ receptors.[9b] In order to overcome the long scanning times required for ^{18}F-fluoro-2-A85380, we have developed agents with faster binding kinetics. These include ^{18}F-nifene (Figure 2, **5**), an agonist and ^{18}F-nifrolidine (Figure 2, **6**), an antagonist, both of which exhibit rapid kinetics in monkey PET studies.[10] Radiolabeling of these radiotracers is carried out with high-specific activity nucleophilic fluorine-18 that is suitable for measuring small concentrations of these receptors. In the case of ^{18}F-nifene,[10b] a fluorine-18 for nitro group exchange was carried out whereas a fluorine-18 for tosylate group exchange was used for ^{18}F-nifrolidine.[10a] Preliminary



^{18}F-FDDNP, **1** ^{11}C-PIB, **2** ^{18}F-FBM, **3**

Figure 1 Radiotracers for β-amyloid plaques and neurofibrillary tangles.



^{18}F-Fluoro-2-A85380, **4** ^{18}F-Nifene, **5** ^{18}F-Nifrolidine, **6**

Figure 2 Radiotracers for nicotinic $\alpha 4\beta 2$ receptors.

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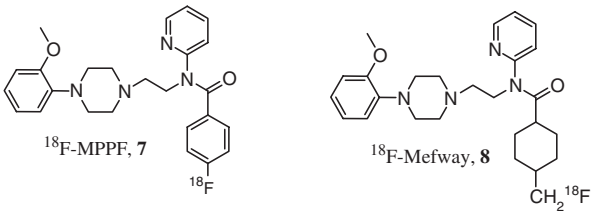

Figure 3 Radiotracers for serotonin 5HT1A receptors.

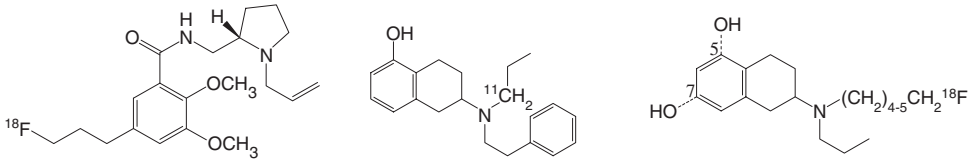

Figure 4 Radiotracers for dopamine D2/D3 receptors.

studies of ^{18}F-nifene in 15- to 18-month-old 3xTg-AD mice indicate a >90% loss of ^{18}F-nifene binding to brain regions such as the thalamus known to be rich in these receptors.[11] This is consistent with the reported decrease in the binding of ^{18}F-fluoro-2-A85380 in AD patients.[9] Further studies with ^{18}F-nifene will allow examination of an age-dependent loss of binding in the 3xTg-AD mice.

3. *Serotonin receptor imaging*: Serotonin 5-HT1A receptors have been implicated in various CNS disorders and are therefore being studied by PET. A loss of serotonin 5HT1A receptors has been seen in AD patients using ^{18}F-MPPF (Figure 3, **7**) in the hippocampal regions, which correlated with poor clinical outcomes.[12] We have recently developed ^{18}F-mefway (Figure 3, **8**) as a relatively more stable, high-affinity serotonin 5-HT1A receptor PET imaging agent.[13] Although ^{18}F-MPPF allows the visualization of change in the hippocampus, ^{18}F-mefway provides greater hippocampus to cerebellum ratios (2–3 for ^{18}F-MPPF and 10 for ^{18}F-mefway in monkeys).[12,13] This higher ratio of ^{18}F-mefway may have the ability to detect change in 5HT1A receptors in AD patients as well as patients with mild cognitive impairment (MCI) with greater sensitivity.

4. *Dopamine receptor imaging*: In recent years, dopamine D2/D3 receptors have been imaged in receptor-rich brain regions such as the striatum and in regions outside the striatum, referred to as extrastriatum. High-affinity, selective agents such as ^{18}F-fallypride (Figure 4, **9**) have been used to quantitatively measure small concentrations of extrastriatal receptors located in the thalamus, amygdala, temporal cortex and other regions.[14a] Using ^{18}F-fallypride, a loss in extrastriatal D2/D3 receptors has been found in drug-naive schizophrenics.[14b] Loss of dopamine D2/D3 receptors in the temporal cortex has been observed in postmortem AD.[14c] This loss of D2/D3 receptors has been implicated in cognitive decline of AD. The study of AD transgenic mice models using ^{18}F-fallypride is currently underway in our laboratory. The longer half-life of fluorine-18 (109.8 min) in ^{18}F-fallypride allows for the measurement of both, high and low D2/D3 receptor concentration regions of the entire brain across several species (mouse, rat, monkey and humans). The chemical structure of fallypride (Figure 4, **9**) also makes it suitable to be radiolabeled with the shorter half-life carbon-11 (20.4 min) which is appropriate for same-day repeat studies of extrastriatal brain regions.[14d]

Dopamine D2/D3 receptors exist in two interconvertible conformational states that are differentiated by their affinity for dopamine. The high-affinity state is considered to be the functional state and therefore more important for detection of anomalies. Antagonists such as ^{18}F-fallypride do not distinguish between the two affinity states. Therefore, a significant amount of effort is currently underway to develop methods to study only the high-affinity state of D2/D3 receptors using agonists. Our efforts have led to the development of several moderate affinity and high-affinity agonists such as ^{11}C-PPHT (Figure 4, **10**) and ^{18}F-5-OH-FPPAT (Figure 4, **11**).[15a,b] The position of the hydroxyl group in ^{18}F-5-OH-FPPAT (Figure 4, **11**) can determine selectivity towards D2 or D3 receptors. In our efforts to target dopamine D3 receptors, the 7-hydroxy derivatives are

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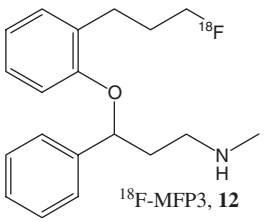
also currently being explored.[15c] The use of agonists along with ^{18}F-fallypride will provide a greater understanding of the status of these receptors in AD.

5. *Norepinephrine transporter imaging*: The NET is responsible for the reuptake of the neurotransmitter, norepinephrine (NE), and regulating the synaptic NE concentration in the brain. It can be found presynaptically to the noradrenergic neurons in high densities in the hippocampus, thalamus, and especially the locus coeruleus, from which the NET pathway is projected. Decreased NET concentration is associated with psychiatric and neurological disorders such as depression and AD.[16a] Degeneration of the locus coeruleus, a region enriched in NE cell bodies, has been observed in AD. We have developed (*R*)-*N*-methyl-3-((^{18}F-3′-fluoropropyl)phenoxy)-3-phenylpropanamine (^{18}F-MFP3, **12**) which is currently being evaluated as a potential PET imaging agent for NET in rat studies.[16b]



^{18}F-MFP3, **12**

6. *Acetylcholine measurements*: Acetylcholinesterase inhibitors are currently the major class of approved drugs used to treat the symptoms of AD, and the putative mode of action is to increase acetylcholine levels.[17a] In order to evaluate efficacy of these drugs, the ability to measure alterations in brain acetylcholine levels during treatment will be valuable. This will assist in the overall management of treatment, minimize side effects, and allow the choice of appropriate therapy. We have evaluated competition of acetylcholine with nicotinic $\alpha4\beta2$ receptor PET agonist radiotracer, ^{18}F-nifene in the presence of two AD drugs, physostigmine and galantamine.[17b] Using *in vitro* studies, both physostigmine and galantamine reduced the binding of ^{18}F-nifene, suggesting competition with acetylcholine. These preliminary results suggest that ^{18}F-nifene PET studies have promise for assessing AD drug effects *in vivo*.

Conclusion

We have prepared PET radiotracers for five different biological processes that are implicated in AD. Our preliminary results using transgenic mice models suggest that a comprehensive, multi-target approach may be valuable in unraveling the biochemical processes of this neuro-degenerative disease. This approach may then be applied to improve the quality of life in AD.

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