Molecular imaging agents for detection of β-amyloid plaques in Alzheimer’s disease
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ABSTRACT: The formation of amyloid structures is a neuropathological feature that characterizes several neurodegenerative disorders, such as Alzheimer’s and Parkinson’s disease. Up to now, the definitive diagnosis of these diseases can only be accomplished by immunostaining of post mortem brain tissues with dyes such Thioflavin T and congo red. Aiming at early in vivo diagnosis of Alzheimer’s disease (AD), several amyloid-avid radioprobes have been developed for β-amyloid imaging by positron emission tomography (PET) and single-photon emission computed tomography (SPECT). The aim of this paper is to present a perspective of the available amyloid imaging agents, special those that have been selected for clinical trials and are at the different stages of the US Food and Drugs Administration (FDA) approval.

Keywords: Alzheimer’s disease, β-Amyloid aggregation, molecular imaging, molecular probes.

Introduction
Alzheimer’s disease (AD) is a neurodegenerative disorder that affects millions of people worldwide¹. The impact in the public health is considerable, with tendency to increase as the population gets older. The most common symptoms of AD are decline in the cognitive functions, irreversible memory loss, disorientation and language impairment. AD diagnosis is based mainly on the patient’s history and on neuropsychological tests. However, the overlapping of early AD symptoms with normal signs of aging dificults such diagnosis. Histopathologically, AD is characterized by the presence of senile plaques containing β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) containing highly phosphorylated tau protein. Currently, the accurate diagnosis of AD is only possible post mortem after confirmation of extracellular Aβ deposits and NFTs, through histopathological studies using dyes such thioflavin T (ThT) and congo red (CR)².

The molecular processes underlying the pathology are still unknown, however it is thought that the Aβ deposits accumulate before the onset of the disease³. Aβ is a soluble extracellular peptide composed by 40 (Aβ₁⁻⁴₀) or 42 (Aβ₁⁻⁴₂) aminoacids, which is formed from transmembrane amyloid-precursor protein (APP) by the action of β and γ secretases⁴. Thus, in vivo imaging agents that can specifically demonstrate the location and density of Aβ deposits...
in AD brain will be useful for an early and conclusive diagnosis of AD (cf. Figure 1). Moreover, these agents will help on the finding and monitorization of novel AD therapies, especially the ones based on the dissolution of the Aβ plaques. Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are among the best suited molecular imaging modalities to achieve such a goal.

**Design of Aβ imaging agents**

In the past few years, several compounds have been designed to interact with the oligomeric and fibrillar forms of the Aβ peptide for its *in vivo* detection\(^5-9\). Those compounds are essentially small, aromatic and heteroaromatic molecules. Their planarity allows for the insertion into the β sheet structure of Aβ plaques, ensuring good binding affinity. A common requisite for these compounds is the ability to cross the blood brain barrier (BBB) to reach the intracerebral target. A good radiotracer for *in vivo* imaging of Aβ plaques by PET or SPECT must have a high initial brain uptake and a fast washout from the normal brain, to ensure a good target/non-target ratio. The design of these aromatic and planar compounds for the targeting of Aβ plaques have been based mainly on the highly conjugated system present in the structures of the ThT and CR dyes. So far, the most promising SPECT and PET radioprobes for *in vivo* imaging of Aβ plaques are compounds containing the gamma emitters \(^{123/125}\text{I}\) and the positron emitters \(^{11}\text{C}\) or \(^{18}\text{F}\), respectively (cf. Figure 2)\(^9\). Although \(^{99}\text{Tc}\) offers several advantages for SPECT imaging, the design of \(^{99}\text{Tc}\)-based radiopharmaceuticals usually requires a bifunctional chelator (BFC) for the metal complexation. Conjugation of BFCs to amyloid-avid molecules produces constructs with limited BBB permeability and therefore unsuitable for *in vivo* application.

**Figure 1:** *In vivo* interaction of an imaging probe with cerebral Aβ plaques.

**Figure 2:** Chemical structures of relevant Aβ imaging agents.

1 \((^{123/125}\text{I})\text{-IMPY})

2 \((^{18}\text{F})\text{-FDDNP})

3 \((^{11}\text{C})\text{-P}(!)\text{B})

4 \((^{18}\text{F})\text{-Flutemetamol})

5 \((^{18}\text{F})\text{-Florbetaben})

6 \((^{18}\text{F})\text{-Florbetapir})
Relevant Radiolabeled Aβ imaging probes

Although there are more SPECT than PET scanners, the same is not true with respect to agents for amyloid imaging. Among the SPECT amyloid imaging, the 123I-IMPY (cf. Figure 2[1]) has shown up as the most promising, while more progress has been observed in the development of PET amyloid imaging radiopharmaceuticals. 123I-IMPY displayed selective binding to Aβ plaque ex vivo in autoradiographic experiments using mice AD model (PSAPP)11. However the signal-to-noise ratio for plaque labelling is not ideal, maybe due to the fast clearance from the brain and plasma observed in AD and normal subjects12.

The compound 18F-FDDNP (cf. Figure 2[2]) was the first PET probe sucessfully developed for in vivo molecular imaging of Aβ plaques13. However, PET imaging showed that 18F-FDDNP labels both Aβ plaques and NFTs in the brain of AD, and thus is not selective for measuring Aβ deposits load in the AD brain. Also, its excessive lipophility (log P = 3.92) contributed for high non-specific binding in normal mice brain14. The “Pittsburgh compound B” (11C-PIB) (cf. Figure 2[3]) is one of the best characterized PET imaging agent for Aβ plaques in the brain. It showed excellent initial brain uptake and a high binding affinity to Aβ plaque (Kᵢ = 0.87 ± 0.18 nM)15. In AD patients, 11C-PIB retention, which was increased in the cortical areas, correlated inversely with cerebral glucose metabolism determined with 18F-fluorodeoxyglucose (18F-FDG) (cf. Figure 3)16. Since then, other studies in thousands of AD patients have validated the usefulness of 11C-PIB as a PET Aβ imaging probe17-20. However, the short half-life of 11C (t₁/₂ = 20 min) limits the clinical use of 11C-PIB to centers with an on-site cyclotron. Such limitation prompted several authors to search for alternative amyloid-binding radiopharmaceuticals labelled with longer lived fluorine-18 (t₁/₂ = 110 min). 18F-Flutemetamol (GE-067) (cf. Figure 2[4]) is very similar to PIB, except that it has an 18F-tag instead of 11C. 18F-Flutemetamol binding reflects Aβ deposits load in post mortem brain tissue. 18F-Flutemetamol is comparable to 11C-PiB in its ability to detect brain Aβ pathology in AD living patients21. Biopsy and autopsy studies showed that 18F-flutemetamol has a high specificity and sensitivity in the detection of Aβ deposits in the brain22. Final Phase III data showed a strong concordance between 18F-Flutemetamol PET imaging and Aβ pathology (cf. Figure 4)23. A New Drug Application (NDA) was submitted to the US Food and Drug Application (FDA) and to European Medicine Agency (EMA) for the use of 18F-flutemetamol in the visual detection of Aβ burden in adult patients suspected of AD24.

The tracers 18F-florbetapen and 18F-florbetapir (cf. Figure 2[5-6]) were also found to display high-affinity binding to Aβ plaques with Ki < 10 nM. Thanks to the pyridine ring in florbetapir, this tracer is less lipophilic than florbetapen. Nonetheless their non-specific binding in white matter is higher than that of 11C-PIB25-26. Clinical studies with 18F-florbetapir demonstrated a strong correlation between in vivo amyloid PET imaging and its post mortem histopathological binding26. Also, 18F-florbetapir-PET/MR studies correlated positively the anatomic data with the localization of 18F-florbetapir retention in the white and gray matter often affected by AD. Clinical interpretation of 18F-florbetapir PET relies upon assessment of gray-white differentiation, with negative studies showing higher activity in the white matter than in the cerebral cortex (cf. Figure 5A) and positive studies showing loss of gray-white contrast due to the tracer binding to beta-amyloid plaques in the cerebral cortex (cf. Figure 5B)27. 18F-Florbetapir has recently been approved by the FDA for clinical use28. Nonetheless, other amyloid PET tracers are in late phase clinical trials and may soon become clinically available.

Figure 3: 11C-PIB standardized uptake value (SUV) and 18FDG rCMRglc images in AD patients and healthy control (HC) subjects13. Reproduced by permission of John Wiley and Sons.

Figure 4: 18F-Flutemetamol images in normal volunteers and in AD patients.
Figure 5: Amyloid imaging with 18F-florbetapir. A) Normal control subject with no-to-sparse Aβ plaques. B) Positive PET/MRI study, consistent with moderate to frequent Aβ plaques20.

Conclusions

The 11C-PIB, 18F-flutemetamol, 18F-florbetapen, and 18F-florbetapir have been well studied in humans as amyloid imaging agents. The imaging performance of these four PET tracers is comparable with high retention in cortical regions, providing all of them good contrast with non-target regions. Despite being the best studied, 11C-PIB has not been yet approved by the FDA, while 18F-flutemetamol is pending FDA and EMA approval. So far, the only amyloid PET tracer authorized by the FDA is the 18F-florbetapir (Amyvid) for brain imaging of cognitively impaired adults undergoing evaluation of AD28.

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References


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