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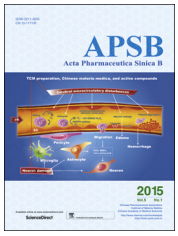

REVIEW

Near-infrared fluorescent probes for imaging of amyloid plaques in Alzheimer's disease

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Abstract One of the early pathological hallmarks of Alzheimer's disease (AD) is the deposition of amyloid-β (Aβ) plaques in the brain. There has been a tremendous interest in the development of Aβ plaques imaging probes for early diagnosis of AD in the past decades. Optical imaging, particularly near-infrared fluorescence (NIRF) imaging, has emerged as a safe, low cost, real-time, and widely available technique, providing an attractive approach for *in vivo* detection of Aβ plaques among many different imaging techniques. In this review, we provide a brief overview of the state-of-the-art development of NIRF Aβ probes and their *in vitro* and *in vivo* applications with special focus on design strategies and optical, binding, and brain-kinetic properties.

Abbreviations: Aβ, amyloid-β; Ach, acetylcholine; AD, Alzheimer's disease; APP, amyloid peptide precursor; BAP, BODIPY-based Ab imaging probe; BBB, blood-brain barrier; Cy, cyanine dyes; ICG, indocyanine green dyes; MRI, magnetic resonance imaging; NIR, near-infrared; NIRF, near-infrared fluorescence; PET, positron emission tomography; ROS, reactive oxygen species; SPECT, single photon emission computed tomography

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1. Introduction

Alzheimer's disease (AD) is the most common type of dementia among older people, affecting approximately 35 million people worldwide, with 5 million new cases every year[1]. Clinical symptoms of AD include progressive cognitive decline, irreversible memory loss, disorientation, language impairment, and emotional instability[1]. The dilemma places significant mental, social and economic burdens on patients, families, and communities[1]. Unfortunately, there are no currently effective treatments available to reverse or stop the progress of this devastating disease, primarily due to difficulties in identification of disease etiology[2–4].

Several pathological hallmarks of this disease have been identified, namely, the deposition of amyloid-β (Aβ) plaques and neurofibrillary tangles, elevated reactive oxygen species (ROS), imbalanced metal ion (*e.g.*, Cu, Fe, and Zn) homeostasis, and decreased brain acetylcholine (Ach) levels. Three major theories have been proposed to explain these pathological hallmarks: amyloid cascade[3,5,6], oxidative stress[7,8], and the metal ion hypotheses[7]. The amyloid cascade hypothesis is currently the prevailing one. It is believed that the formation Aβ plaques arises from aggregation of peptides Aβ_{40} and Aβ_{42}, and is the initial event in the pathogenesis of the AD. Aβ_{40} and Aβ_{42} are degradation products of amyloid peptide precursor (APP), generated from cleavage by β- and γ-secretases. These cleaved peptides have a tendency to aggregate into different Aβ species such as dimers, oligomers, fibrils, and plaques, and may also interact with metal ions and produce ROS, with subsequent neuronal atrophy and death[4]. Regardless of the nature of the intertwined toxicological pathways induced by Aβ aggregates, it is widely accepted that the formation of Aβ plaques precedes the clinical symptoms of AD. Therefore, they are excellent diagnostic and predictive biomarkers for the early detection of AD[5,6,9]. Moreover, the current clinical diagnosis of AD is primarily based upon family and patient's medical history as well as neurological and neuropsychological observations. Thus, the diagnosis is often inaccurate. Confirmative AD diagnosis can only be made through postmortem histopathological examination of brain Aβ plaques. There exists a great and urgent need to develop non-invasive and accurate probes for Aβ plaques to improve the current diagnosis of AD. Such probes will also be useful in monitoring disease progression and effectiveness of new AD treatments.

In the past decade, significant advances have been made in the design of molecular probes for specific labeling, detection, imaging of Aβ plaques both *in vitro* and *in vivo*. A number of different imaging modalities and approaches have been applied, including magnetic resonance imaging (MRI)[10–14], positron emission tomography (PET)[15–19], single photon emission computed tomography (SPECT)[20–24], and optical imaging techniques[25]. MRI based approaches suffer from low resolution since the size of Aβ plaques typically range from 20 to 60 μm, while only larger plaques (>50 μm) are detectable[26]. Compared with MRI, radiolabeled PET and SPECT probes are more sensitive methods. Many probes, such as [^{11}C]PIB[27,28], [^{11}C]SB-13[29,30], [^{11}C]AZD2184[31], [^{18}F]FPIB[32], [^{18}F]AZD4694[33,34], [^{18}F]FDDNP[35,36], [^{18}F]AV-1[37-39], [^{18}F]AV-45[40–42] and [^{123}I]IMPY[20], have advanced in clinical trials. PET-based probes are more promising in terms of their translational applications. Three PET probes [^{18}F]FPIB (Vizamyl^{TM}), [^{18}F]AV-45 (Amyvid^{TM}) and [^{18}F]AV-1 (Neuraceq^{TM}) were recently approved by the FDA. The clinical diagnostic utility of these PET imaging agents is limited: they cannot be used to confirmatively diagnose AD, only to support other diagnostic criteria[43]. Furthermore, the use of PET probes is limited by high cost and narrow availability, since generation of these probes needs specialized facilities that have a cyclotron for the generation of short-lived radionuclides (*e.g.*, [^{11}C], $t_{1/2}$=20 min and [^{18}F], $t_{1/2}$=110 min) and an automated synthetic unit to produce radiolabelled probes. Compared with PET, SPECT has broader availability and lower cost as a routine diagnosis method due to the use of easily-generated radionuclides with longer half-lives (*e.g.*, [^{125}I], $t_{1/2}$=60.1 d, [^{123}I], $t_{1/2}$=13.2 h, and [^{99m}Tc], $t_{1/2}$=6.0 h). Current SPECT-based probes either have relatively high background for the radioiodinated probes due to high lipophilicity and nonspecific binding or have poor blood-brain barrier (BBB) penetration in the case of ^{99m}Tc-labeled SPECT probes. Only one SPECT probe, [^{123}I]IMPY, has advanced in clinical trials. In general, radionuclear-based imaging modalities PET and SPECT are limited by high cost, radiation exposure, and single signal readout.

In contrast to the radionuclear-based imaging techniques, optical imaging modalities are rather inexpensive; important merits include nonradioactive, real-time imaging with the option of multi-targets tracing *in vitro* and *in vivo*, wide availability, and high-resolution imaging depending on the specific technique used[44–47]. For *in vivo* applications, in order to avoid absorption and background autofluorescence and scattering of biological molecules, probe fluorescence emission wavelength in the near-infrared (NIR) region between 650 and 900 nm is advantageous so that one can achieve an optimal penetration depth and high sensitivity. Therefore, NIR fluorescence imaging has emerged as an attractive alternative to PET/SPECT and MRI techniques, and may provide a solution for the early diagnosis of AD. In the following sections, we discuss challenges and design strategies associated with the development of NIRF Aβ probes for *in vivo* applications, followed by a list of current reported probes and their optical, binding and brain-kinetic properties, as well as *in vitro* and/or *in vivo* studies (Table 1).

2. Challenges in developing NIRF Aβ probes

A number of NIR fluorophores such as cyanine dyes (Cy7), indocyanine green dyes (ICG), alexa fluor dyes (660–790 nm), and SRfluor dyes have been developed and employed for *in vivo* applications; many of them are commercially available[47]. Nonetheless, these known NIR fluorophores have large molecular weight and intrinsic charges. They are likely to be unsuitable for labeling Aβ plaques in the brain because of their limited BBB permeability. In order to use a fluorophore for *in vivo* brain Aβ imaging, several criteria are required[48–50]: (1) a suitable wavelength of excitation and emission within the NIR range (650−900 nm); (2) high BBB permeability (log*P* values between 2 and 3.5, or *c*log*P*<5.0 are considered optimal[51,52]); (3) high affinity for specific labeling of the Aβ plaques in the brain with low nonspecific binding to other proteins; (4) rapid clearance of the unbound dye from the brain; and (5) significant changes in the probe fluorescence properties upon binding to Aβ plaques. It is challenging to design probes meeting all the requirements. First, many NIR fluorophores are often highly-conjugated structures with molecular weight over 600 Da, while a small and compact scaffold with molecular weight less than 600 Da is required for NIRF Aβ probes. Secondly, the probes should have balanced lipophilicity to ensure good BBB penetration and avoid nonspecific binding. Moreover, high affinity and significant fluorescence property changes require fluorophore scaffolds which are challenging to design. Ultimately, it is difficult to predict *in vivo* properties of a designed NIRF probe before synthesis and testing.