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Gadolinium-based contrast agents targeted to amyloid aggregates for the early diagnosis of Alzheimer's disease by MRI

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ABSTRACT

While important efforts were made in the development of positron emission tomography (PET) tracers for the *in vivo* molecular diagnosis of Alzheimer's disease, very few investigations to develop magnetic resonance imaging (MRI) probes were performed. Here, a new generation of Gd(III)-based contrast agents (CAs) is proposed to detect the amyloid β-protein (Aβ) aggregates by MRI, one of the earliest biological hallmarks of the pathology. A building block strategy was used to synthesize a library of 16 CAs to investigate structure–activity relationships (SARs) on physicochemical properties and binding affinity for the Aβ aggregates. Three types of blocks were used to modulate the CA structures: (i) the Gd(III) chelates (Gd(III)-DOTA and Gd(III)-PCTA), (ii) the biovectors (2-arylbenzothiazole, 2-arylbenzoxazole and stilbene derivatives) and (iii) the linkers (neutrals, positives and negatives with several lengths). These investigations revealed unexpected SARs and a difficulty of these probes to cross the blood–brain barrier (BBB). General insights for the development of Gd(III)-based CAs to detect the Aβ aggregates are described.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder responsible for 60%–70% of dementia cases. About 6% of the population aged 65 and older suffer from dementia, which represented 36 million people worldwide in 2010 with a total societal cost estimated to be US$ 604 billion. The number of AD patients is believed to double every 20 years to potentially reach 115 million by 2050 [1]. AD has both societal and economical impacts on our society and is expected to become one of the major health care problems for industrialized countries in the future [2]. As a matter of fact, the research for a treatment and an early diagnosis of AD, with *in vivo* imaging of amyloid plaques as the most promising technique [3], appears today as a priority [4]

In the last 20 years, the research on AD focused on the development of different therapeutic approaches and diagnostic techniques to detect the pathology at an early stage [5]. However, to date the definitive diagnosis of AD can be performed *post-mortem* only [6]. An early diagnosis of AD would allow not only an intervention with future disease-modifying therapies right from the start of the disease, but also a relevant follow-up of drugs tested [7]. The neuropathological hallmarks of AD are intracellular neurofibrillary tangles, caused by the hyperphosphorylation of tau proteins, and extracellular neuritic plaques, caused by the aggregation

Abbreviations: BAT, bis-amine-bis-thiol; BTA, 2-(4-*N*-methylaminophenyl)benzothiazole; CA, contrast agent; CMC, critic micelle concentration; CV, cone voltage; DIEA, *N*,*N*-diisopropylethylamine; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; EDCI HCl, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride; FDG, fluorodesoxyglucose; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOBt, 1-hydroxybenzotriazole; IBOX, 2-(4-dimethylaminophenyl)-6-iodobenzoxazole; ICP-AES, inductively coupled plasma-atomic emission spectroscopy; ICP-MS, inductively coupled plasma-mass spectroscopy; IMPY, 6-iodo-2-(4′-dimethylamino)-phenyl-imidazo[1,2-a]pyridine; LRMS, low-resolution mass spectroscopy; MAMA, monoamide-monoaminedithiol; MCI, mild cognitive impairment; MION, monocristalline iron oxide nanoparticle; NHS, *N*-hydroxysuccinimide; PCTA, 3,6,9,15-tetraaza bicyclo[9.3.1]-pentadeca-1(15),11,13-triene-3,6,9-triacetic acid; PIB, Pittsburgh Compound-B; r_1, longitudinal relaxivity; r_2, transversal relaxivity; SB, stilbene; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time; th-T, thioflavin-T; UBTA, *N*,*N*′-bis[2-mercapto-2-methylpropyl]2-aminobenzylamine; USPIO, ultrasmall superparamagnetic iron oxide.

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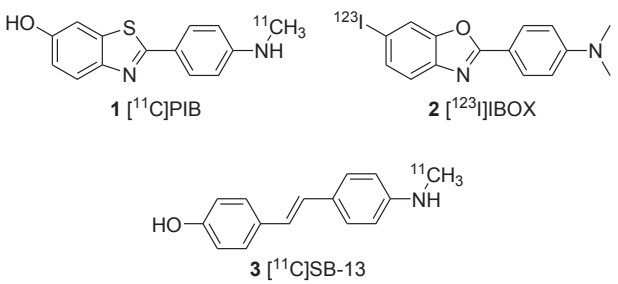
of amyloid β-protein (Aβ) fibrils, accompanied with neurotransmitter deficits. The "amyloid hypothesis" [8], today accepted by a large part of the scientific community, suggests that Aβ plaques appear in the brain 10–20 years before the first clinical symptoms of AD. The Aβ aggregates are believed to be one of the most relevant hallmarks along the AD evolution process [9,10]. *In vivo* methods for amyloid imaging [11], together with cerebrospinal fluid biomarkers, as well as functional monitoring of the brain (glucose metabolism with [^{18}F]fluorodesoxyglucose ([^{18}F]FDG) or neurotransmitter activity) [10,12], are expected to have a high potential to specifically diagnose AD patients at a very early stage. During the last 10 years, various molecular imaging radiolabeled tracers have been reported for the detection of amyloid deposits by single-photon emission computed tomography (SPECT) and positron emission tomography (PET) and some are currently assessed on clinical trials [10,13–15]. Florbetapir-^{18}F has been the first amyloid specific ^{18}F PET tracer approved by the Food and Drug Administration (FDA) in 2012 for adults being evaluated for AD or other cognitive disorders [16]. The detection of amyloid deposits in human with the Pittsburgh Compound-B ([^{11}C]PIB) and Florbetapir-^{18}F by PET imaging showed to be able to differentiate a population at risk of developing AD from mild cognitive impairment (MCI) patients [17–19]. Interestingly, around 20–40% of asymptomatic controls showed a [^{11}C]PIB retention [20–24], which suggests that this compound might be an efficient tracer for AD preclinical diagnosis since the major growth of amyloid burden seems to occur at this stage of the disease [25–27].

The development of probes for the detection of the AD hallmarks by nuclear magnetic resonance imaging (MRI) has been much less successful, mainly due to the lower sensitivity of this technique compared to PET and SPECT. To date, magnetic probes have been studied only at a pre-clinical stage. However, the advantages provided by MRI over radionuclide-based imaging techniques encouraged us to investigate in this direction. Compared to SPECT or PET, MRI technique does not require the injection of radioactive probes, has better resolution (200 μm–50 μm for clinical and research magnets respectively) and provides anatomic information which could be relevant for quantifying amyloid deposits and characterising precisely the deposition areas [28,29]. The high resolution achievable by MRI makes the detection of amyloid lesions possible both in humans and animals, which is also critical for the evaluation of new treatments against AD at early stages. Moreover, the lower cost, wider availability and the absence of irradiation of the MRI exams afford important advantages.

Contrary to radionuclide-based imaging techniques such as PET and SPECT, MRI does not allow the direct detection of the CA. Indeed the signal detected by MRI is the relaxation rate enhancement of the spins of the water protons in close proximity to the paramagnetic Gd(III) lanthanide. This complex interaction usually prevents the correlation of the local Gd(III) concentration with the MRI signal variation detected in biological media because of the influence of the surrounding of the water protons on the effect of the Gd(III) lanthanide. Nevertheless, specialists agree that the detection of Gd^{3+} ions at concentration of 1–10 μM in the brain could be achieved by using Gd(III)-based MRI CAs with relaxivity equivalent to commercial MRI CAs (i.e. 3 mM^{-1} s^{-1}.Gd^{-1}) and clinical MRI equipment. However, both the density of the targeted site and the relaxivity of the CA bound to the targeted site influence the local concentration of Gd^{3+} ions required for MRI detection. For instance, S. Aime et al. estimate that $4 \pm 1 \times 10^7$ Gd^{3+} ions per cell are required to reach a concentration sufficient to be detected *in vivo*, leading to a local Gd^{3+} ion concentration of 2 μM [30]. C. Corot et al. reported a folate receptor targeting Gd(III)-based CA which requires a local Gd^{3+} ion concentration of 0.92 μM to be detected *in vitro* thanks to its high longitudinal relaxivity (r_1 = 25 mM^{-1} s^{-1}.Gd^{-1}, 2.35 T, 37 °C) [31]. The density of Aβ peptides in AD frontal cortex brain was determined at 1–3 μM [32] and the binding stoichiometry of CAs to Aβ in AD brain is close to 1:1 under saturating conditions [33]. Considering that a relaxivity enhancement of the CAs (~×2 at 60 MHz, 37 °C) is expected after binding to the amyloid aggregates, thanks to an increase of the rotational correlation time [34], CAs with relaxivities around 10 ± 5 mM^{-1} s^{-1} have a great potential to dectect amyloid aggregates *in vivo* if the targeted sites can be reached.

Several Gd-based CA have been reported for the detection of amyloid aggregates in mice by MRI. The Gd^{III} diethylenetriaminepentaacetic acid (Gd(III)-DTPA) complex was first attached to the N-terminal of the peptide $Aβ_{1-40}$ [35]. Amyloid deposits could be detected *in vivo* with 7 T μMRI and T_2* weighted images after transient opening of the blood–brain barrier (BBB) with mannitol. Several ways to enhance BBB permeability of the probes were investigated further. The introduction of putrescine moieties on the peptide $Aβ_{1-40}$ [36] and on the truncated derivative $Aβ_{1-30}$ [37] by modification on glutaric acid and aspartic acid residues were investigated by intravenous injections in APP/PS1 transgenic mice and *ex vivo* detection of the probes. The peptide $Aβ_{1-30}$ elongated with 6 lysine residues was attached to the Gd(III)-DTPA chelate and the CA was dissolved in a mannitol containing buffer before intracarotid injection in a Tg2576 mouse and *in vivo* detection with 7 T μMRI and T_2* weighted images [38]. Even if these structure modifications improved BBB permeability without affecting the binding on amyloid deposits, these probes do not cross the BBB enough to be detected *in vivo* by MRI when adjuvants such as mannitol is not used. Recently, a PIB derivative labelled with Gd(III), ^{111}In(III) and Eu(III) was reported [39]. In spite of a low binding affinity for the $Aβ_{1-40}$ aggregates (K_d = 180 μM), the Eu(III) derivative CA showed to successfully label the amyloid deposits in *post-mortem* human brain tissues of AD patients. However, the BBB permeability of the CA was expected to be insufficient for *in vivo* MRI detection. The solubility and the parameters determining the relaxivity of the Gd(III) derivatives was successfully tuned by modifying the linker between the Gd(III) chelate and the PIB derivative [34]. Iron oxide particles such as monocristalline iron oxide nanoparticles [35] (MIONs) and utrasmall superparamagnetic iron oxide (USPIO) nanoparticles [40], labelled with $Aβ_{1-40}$ and $Aβ_{1-42}$ respectively, have been investigated with similar approaches and were able to bind the amyloid aggregates *in vivo* after injection in APP/PS1 transgenic AD mice with 15% mannitol in phosphate buffer saline (PBS). Similarly, these probes validated the possibility to detect amyloid *in vivo* by MRI but their use was limited by a poor BBB permeability. Introduction of PEG on the surface of $Aβ_{1-42}$ modified USPIO significantly improved the BBB permeability of the CA [41]. After intravanous injection of this CA without adjuvant, Aβ aggregates were clearly detected *ex vivo* (overnight scanning, 7 T μMRI, T_2* weighted images). However, *in vivo* detection was more



1 [^{11}C]PIB **2** [^{123}I]IBOX **3** [^{11}C]SB-13

Fig. 1. Chemical structures of amyloid specific ligands [^{11}C]PIB (**1**), [^{123}I]IBOX (**2**) and [^{11}C]SB-13 (**3**).