^{18}F-LABELED PEG STILBENE DERIVATIVES AS PET IMAGING AGENTS FOR β-AMYLOID PLAQUES IN THE BRAIN

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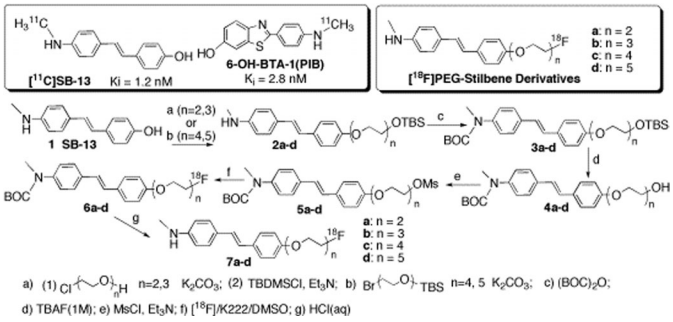
Accumulation of β-amyloid (Aβ) plaques in the brain is considered as one of the key neuropathology associated with Alzheimer's disease (AD). Thus, development of imaging agents, especially positron emission tomography (PET) agents targeting Aβ plaques, will be useful for diagnosis and monitoring disease progression. Previously, a stilbene derivative, [^{11}C]SB-13, showed promise in detecting senile plaques present in AD patients[1]. The short half-life (20 min) of C-11, however, may limit the usefulness of [^{11}C]SB-13[1] or [^{11}C]PIB[2] for a wide spread application; comparable ^{18}F labeled agents may supplant the clinical needs. Reported herein is the preparation of ^{18}F labeled polyethylene glycol (PEG) stilbene derivatives designed to target Aβ plaques in the brain. In these series of compounds, ^{18}F is linked to the stilbene through a PEG chain, of which the number of ethoxy groups ranges from 2 to 5. It is known that adding PEG groups will lower the lipophilicity and improve bioavailability.



The syntheses of ^{18}F labeled PEG stilbene derivatives are achieved by the scheme shown above. All of the fluorinated stilbenes displayed high binding affinities in binding assay using postmortem AD brain homogenates (Ki = 3.0-7.0 nM). Labeling with ^{18}F was successfully performed by a substitution of the mesylate group of **5a-d** with [^{18}F]fluoride followed by HCl(aq) to remove the BOC protection group giving the target compounds [^{18}F]**7a-d** (EOS, specific activity, 0.9-1.5 Ci/μmol; radiochemical purity >98%). *In vivo* biodistribution of these novel ^{18}F ligands in normal mice exhibited excellent brain penetrations and rapid washouts after an iv injection (6.6-8.1 and 1.2-2.6 %dose/g at 2 min and 60 min, respectively). Autoradiography of postmortem AD brain sections of [^{18}F]**7a-d** confirmed the specific signal due to the presence of Aβ plaques. In addition, *in vivo* plaque labeling can be clearly demonstrated with these ^{18}F labeled agents in transgenic mice (Tg2576), a useful animal model for Alzheimer's disease. In conclusion, the preliminary results strongly suggest these fluorinated PEG stilbene derivatives are suitable candidates as Aβ plaque imaging agents for studying patients with Alzheimer's disease.

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Keywords: PET Imaging, Alzheimer's Disease, F-18 Labeling

SYNTHESIS AND EVALUATION OF ^{18}F-LABELED FIMPYD AS A PET IMAGING AGENT FOR β-AMYLOID PLAQUES

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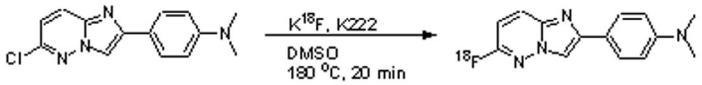
Introduction.The accumulation of β-amyloid (Aβ) plaques in the brain is considered one of the most significant factors in pathogenesis of Alzheimer's disease (AD). Therefore, developing radiolabeled amyloid-specific imaging agents would potentially provide a tool to evaluate individuals with the earliest stage of the neuropathologic alterations of AD and would be useful for evaluating the efficacy of new antiamyloid therapies. Here we report the synthesis and evaluation of a novel ^{18}F-labeled thioflavin derivative, 2-(4′-dimethylaminophenyl)-6-fluoroimidazo[1,2-b]pyridazine,as a prospective PET radioligand for Aβ.

Experimental. Reference FIMPYD was synthesized by the condensation reaction between the bromoketone and 3-amino-6-fluoropyridazine. Treatment of the chloride precursor, 6-chloro-2-(4′-dimethylaminophenyl)imidazo[1,2-b]pyridazine, with anhydrous [^{18}F]HF, K_{222}, and K_2CO_3 in DMSO at 180 °C for 20 min gave [^{18}F]FIMPYD. The purified radioligand was injected i.v. into normal rhesus monkey followed by sequential scanning for 2 hours using a Concorde MicroPET P4 to determine pharmacokinetics. Autoradiography of postmortem brain sections of human AD patients and controls with [^{18}F]FIMPYD were carried out.



Results. FIMPYP displayed excellent competition for ^{125}I-IMPY binding to human AD brain tissues, with Ki = 87 nm vs Ki = 172 nm (IMPY). The overall radiochemical yield (decay-corrected) was 43%. The $logP_{7.4}$ value was 2.75. MicroPET imaging showed high [^{18}F] uptake in cerebellum (Cer), frontal cortex (FC), and subcortical white matter (SCWM) was obtained between 7 min to 12 min after iv injection. The radioactivity ratio of the peak to 120 min in Cer, FC, and SCWM is 7, 3.7 and 3, respectively. Specific [^{18}F]FIMPYD binding was clearly observed in the cortical gray matter, but not in the white matter of AD cases. Plaque binding was confirmed by immunohistochemical staining. The control sections showed no binding.

Conclusions: The encouraging in vitro and in vivo properties of [^{18}F]FIMPYD support its candidacy as a radioligand for in vivo imaging amyloid deposits.

Keywords: beta-Amyloid Plaque Imaging, Fluorine-18, Alzheimer's Disease

AN *IN VITRO* ASSAY TO EVALUATE β-AMYLOID LIGANDS USING ISOLATED HUMAN AMYLOID PLAQUES OF ALZHEIMER'S DISEASE BRAIN

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Introduction. Determination of the binding affinities of new ligands for beta-amyloid is the first step in selecting candidate radioligands for PET studies in humans. Three types of amyloid plaques have been used to assay ligand binding *in vitro*, namely synthetic aggregates of Abeta[1-40], Abeta[1-42], amyloid plaques from transgenic mice and amyloid plaques from human Alzheimer's disease (AD) brain tissue. Results from the three types and also from different batches of synthetic amyloid plaques vary with respect to binding site concentration [1]. Since the ultimate test for a radioligand is successful application in humans, *in vitro* evaluation using human AD brain tissue is highly appropriate. However, it should be noted that other proteins with similar binding sites might interfere with the binding of the ligand to beta-amyloid plaques. This may then be reflected in a lower percentage of displaceable radioactivity in the binding assay [2]. The use of isolated human amyloid plaques may also help to identify other native binding sites. Here we report our *in vitro* assay using isolated human b-amyloid plaques as the first step in evaluating potential PET radioligands.

Experimental & Results. We surveyed literature methods for isolating human beta-amyloid plaques from brain tissue [3-4] and developed a modified method by combining desirable protocol features. The displacement of [^{3}H]6-OH-BTA-1 by non-radioactive 6-OH-BTA-1 or other ligands resulted in classical displacement curves. Non-radioactive 6-OH-BTA-1 achieved >95% displacement of reference radioligand, indicating that the presence of competing binding sites for the isolated amyloid plaques was negligible (Figure 1). This assay could therefore be used to screen compounds for amyloid binding without interference from other proteins, such as tau-tangles. This displacement curve was also analyzed using a homologous displacement mathematical model to extract the B_{max} of the amyloid plaques (Scheme 1). The ratio between B_{max} and the amount of Ab1-42 monomer measured by ELISA is about 1: 2, somewhat less than that reported previously [1]. The denaturing agents used to aid dissolution of the amyloid plaques may account for the observed difference.

Conclusions. Human b-amyloid plaques were isolated in a highly refined state and used to develop an *in vitro* assay to screen novel ligands for binding to amyloid. Both non-radioactive 6-OH-BTA-1 and other test ligands showed classical displacement curves. The molar ratio between B_{max} and the amount of Abeta[1-42] monomer measured by ELISA is quite close to that reported, indicating that the isolation procedure preserves the binding sites of 6-OH-BTA-1.

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Keywords: In Vitro Assay, beta-Amyloid, Alzheimer's Disease

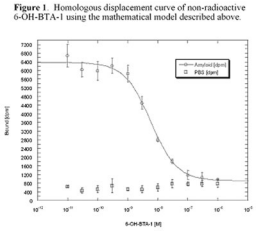

Figure 1. Homologous displacement curve of non-radioactive 6-OH-BTA-1 using the mathematical model described above.

Scheme 1. Single binding site model for homologous displacement.

$[B] = [B]max*[F]/(Kd + [F])$
$[F] = [F1] + [F2]$
$[B] = [B1] + [B2]$
$[B1] * [F2] = [B2] * [F1]$, since B1 + F2 ⇌ B2 + F1

F1 and B1 are radioactive free and bound forms of the ligand. F2 and B2 are added non-radioactive free and bound forms of the ligand

$[B] = [B1] + [B2] = [B1] + [B1] * [F2]/[F1]$
$= [B1] * [F]/[F1]$
$[B1] * [F]/[F1] = [B]max*[F]/(Kd + [F])$
$[B1]/[F1] = [B]max/(Kd + [F])$

$[B1] = [B]max*[F1]/(Kd + [F])$

VISUALIZATION OF β-AMYLOID DEPOSITS IN THE LIVING BRAIN OF A TRIPLE TRANSGENIC RAT MODEL OF β-AMYLOID DEPOSITION USING [^{18}F]FDDNP-MICROPET IMAGING

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Introduction: Several molecular probes targeting pathological lesion found in brains of Alzheimer's disease (AD) patients have been developed in radiolabeled forms suitable for positron emission tomography (PET) and applied for *in vivo* PET scanning of these patients in recent years. Rodent models of AD β-amyloidosis, which develop abundant deposits of diffuse and dense core β-amyloid plaques in the brain cortex provide an excellent opportunity for both validation of these *in vivo* molecular imaging approaches and for validation of new molecular probes. Earlier we have successfully utilized [F-18]FDDNP, a radiofluorinated molecular probe with affinity for "amyloid-like" structures (e.g., β-amyloid and tau deposits) for *in vivo* imaging of brain pathology in AD patients. Here we report the results of successful utilization of [F-18]FDDNP for *in vivo* microPET imaging of β-amyloid in a triple transgenic rat model of brain β-amyloidosis, recently developed by Cephalon, Inc. and Xenogen Biosciences.

Methods: Three triple transgenic rats (15 months old) and two control Sprague-Dawley rats (9 months old) were injected with 0.5 – 1.5 mCi of [F-18]FDDNP and scanned with Concorde Focus microPET camera for sixty minutes. After normalization and FBP reconstruction the resulting dynamic images were used to generate the distribution volume (DV) parametric images using Logan plot graphical analysis with cerebellum as reference region. These parametric images were used for the quantitative analysis.

Results: [F-18]FDDNP-microPET images from the control animals (three experiments, one rat was scanned twice) show almost uniform distribution of low level binding throughout the cortex and cerebellum (*e.g.* DV values for frontal cortex and for hippocampus are 1.04 ± 0.01 and 0.99±0.04, respectively). In contrast, all three transgenic animals (five experiments, two transgenic rats were scanned twice) show the expected increase in binding in cerebral cortex but not in subcortical regions or white matter when compared with cerebellum (*e.g.* frontal cortex DV is 1.32±0.04 (p<0.0005) and hippocampal DV is 1.23±0.05 (p=0.001)) which is consistent with the known distribution of pathology in the brains of these animals. Specificity of cortical [F-18]FDDNP binding was demonstrated by its blockage with three doses (8-mg each) of naproxen starting one day before the microPET scans (two transgenic rats) bringing the cortical binding levels down to the levels observed in control animals. These results are consistent with the previous observations of inhibitory effect of naproxen on [F-18]FDDNP binding to β-amyloid fibrils *in vitro* and in human brain specimens. **Conclusions:** These results demonstrate that [F-18]FDDNP is a very sensitive molecular imaging probe for *in vivo* microPET detection of brain β-amyloid load in the triple transgenic rat model of amyloid deposition thus validating the usefulness of [F-18]FDDNP PET for *in vivo* imaging in AD. These results open the opportunity for longitudinal monitoring of the progression of brain pathology in the living animals and for studying the effectiveness of new therapeutic treatment approaches targeting b-amyloid. The rat model is also a powerful tool for screening and evaluation of new molecular probes for β-amyloid aggregates.

Keywords: Transgenic Model of AD, microPET Imaging, [F-18]FDDNP