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Featured Article

Amyloid tracers binding sites in autosomal dominant and sporadic Alzheimer's disease

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Abstract

Introduction: Amyloid imaging has been integrated into diagnostic criteria for Alzheimer's disease (AD). How amyloid tracers binding differ for different tracer structures and amyloid- β aggregates in autosomal dominant AD (ADAD) and sporadic AD is unclear.

Methods: Binding properties of different amyloid tracers were examined in brain homogenates from six ADAD with APP^{swe}, PS1 M146V, and PS1 E49 mutations, 13 sporadic AD, and 14 control cases.

Results: ³H-PIB, ³H-florbetaben, ³H-AZD2184, and BTA-1 shared a high- and a varying low-affinity binding site in the frontal cortex of sporadic AD. AZD2184 detected another binding site (affinity 33 nM) in the frontal cortex of ADAD. The ³H-AZD2184 and ³H-PIB binding were significantly higher in the striatum of ADAD compared to sporadic AD and control. Polyphenol resveratrol showed strongest inhibition on ³H-AZD2184 binding followed by ³H-florbetaben and minimal on ³H-PIB.

Discussion: This study implies amyloid tracers of different structures detect different sites on amyloid- β fibrils or conformations.

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Keywords:

Alzheimer's disease; Amyloid- β ; Autosomal dominant Alzheimer's disease; Positron emission tomography; Resveratrol; Pittsburgh compound B; AZD2184; Florbetaben

1. Introduction

The rapid development of molecular imaging enables measurement of brain amyloid- β (A β) plaques *in vivo* and facilitates an early detection of AD [1]. From the initial amyloid PET studies with ¹¹C-Pittsburgh compound B (PIB), three PET tracers ¹⁸F-florbetapir, ¹⁸F-florbetaben, and ¹⁸F-flutemetamol have been approved by US Food and Drug Administration and European Medical Association for use in clinical assessment of memory impairment to exclude AD.

Subjects clinically diagnosed with AD without A β plaques at autopsy or PIB PET negative AD patients included in A β immunization trials [2] raise the questions of clinical variants of AD or misdiagnosis of AD. Appropriate use of biomarkers such as A β PET will improve diagnostic accuracy. A β tracers might vary in their binding properties to different A β fibrils and thereby provide new insight into pathophysiological mechanisms and variants of AD. *In silico* modeling studies have suggested six different surface and core binding sites on synthetic A β fibrils similarly using thioflavine-T compound (e.g., PIB), florbetaben, and florbetapir [3–5]. Three binding sites have been demonstrated on synthetic A β fibrils [6]. A multiple-binding site model for A β tracers was

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suggested in AD autopsy brain where florbetapir, florbetaben, and PIB mainly bind to a high-affinity site 1 (nanomolar range), BF-227 mainly with low-affinity to site 3, and FDDNP partly binding to site 2 [7].

Autosomal dominant AD (ADAD) [8] characterized by atypical forms of A β aggregates represents interesting models to study target engagement of A β tracer to fibrillar as well as other A β conformations. Prominent ^{11}C -PIB retentions have been reported in ADAD carrying *PS1* and *APP* mutation, in cortical and particularly subcortical brain regions [9–15]. In two exceptions, *APP*_{Parc} [13] and *APPE* Δ 693 [16], low cortical ^{11}C -PIB retention was reported compared to sporadic AD (sAD) probably due to ring-shaped plaques or oligomeric A β accumulation in the brain.

In the present study, we compared the binding characteristics of A β PET tracers ^3H -PIB, ^3H -AZD2184, ^3H -florbetaben, and methyl- ^3H -BTA-1 in autopsy brain tissues from the frontal cortex and striatum of patients with sAD and ADAD, respectively. An additional third binding site was detected by AZD2184 in the frontal cortex of ADAD patients as opposite to the other A β tracers. High AZD2184 and PIB binding were measured in the striatum of ADAD compared to sAD. In addition, AZD2184 competed in nanomolar range with anti-A β phenol compound resveratrol. The A β binding properties seem to depend both on the chemical structures of A β tracers as well as the conformation of A β aggregates.

2. Methods

2.1. Materials

[^3H]-2-[6-(methylamino)pyridin-3-yl]-1,3-benzothiazol-6-ol (^3H -AZD2184, specific activity (SA) 21.9 Ci/mmol) and AZD2184 were synthesized at the Karolinska Institutet PET Radiochemistry Laboratory, Stockholm, Sweden. [N-methyl- ^3H]-2-(4'-methylaminophenyl)-6-hydroxybenzothiazole (^3H -PIB, SA 85 Ci/mmol) and Methyl- ^3H -BTA-1, (SA 84 Ci/mmol) were synthesized by Quotient Bioresearch, Cardiff, UK. [N-methyl- ^3H]-4-[(E)-2-(4-{2-[2-(2-fluoroethoxy)ethyl]ethoxy}phenyl)ethenyl]-aniline (^3H -florbetaben, SA 63 Ci/mmol) and florbetaben were synthesized by

Bayer Healthcare Pharmaceuticals, Berlin, Germany. 2-(4'-Methylaminophenyl)benzothiazole (BTA-1) and 4,5-trihydroxy-trans-stilbene (resveratrol) were purchased from Sigma Aldrich, MO. Fig. 6 showed the chemical structures of amyloid tracers and resveratrol drawn using ChemDraw Standard 14.0 (CambridgeSoft, Perkin Elmer, MA).

2.2. Sporadic AD, ADAD, and control brain studies

Frontal cortex and striatum (caudate nucleus and putamen) tissues from 13 patients with sAD (mean age, 68.2 ± 8.5 y), clinically diagnosed and confirmed by pathologic examination according to NINCDS-ADRDA criteria, and 14 age-matched controls (mean age 74.1 ± 12.0 y) were obtained at autopsy and provided by the Netherlands Brain Bank, Netherlands Institute for Brain Research, Amsterdam, Netherlands (Table 1). Brain tissues from six ADAD cases including three *APP*_{swe} mutation carriers (mean age 63.3 ± 3.7 y) and one *PS1* M146V mutation carrier (age 48 y) were provided by the Brain Bank at Karolinska Institutet, Stockholm, Sweden. Brain tissues from one *PS1* M146V mutation carrier (age, 43 years) and one *PS1* E49 mutation carrier (age, 66 years) were provided from the Department of Pathology, University of Helsinki, Helsinki, Finland. Permission to use autopsy brain material in experimental procedures was granted by the Regional Human Ethics committee in Stockholm and the Swedish Ministry of Health and Ethics Committee of the Northern Ostrobothnia Hospital District, Finland. Brain regions were dissected following the recommendation of BrainNet Europe for fixation and anatomic dissection (www.brainnet-europe.org).

2.3. Bielschowsky silver staining in ADAD and sporadic Alzheimer's cases

For neuropathological investigations, brain tissues from ADAD and sAD were fixed in buffered 4% formaldehyde for 4–6 weeks, paraffinized, and cut to 5- μm thick sections from the frontal medial gyrus and striatum. The sections were stained in the same laboratory condition by Bielschowsky silver impregnation according to Yamamoto modification [17].

Table 1
Demographic information for sporadic and autosomal dominant Alzheimer's disease cases

Cohort	N	Age (range)	Sex (F/M)	APOE ϵ 4 (0/1/2)	Disease duration (y)	Postmortem delay (h)
sAD	13	68.9 ± 8.5 (58–81)	7/6	3/3/7	6.1 ± 3.2	5.0 ± 0.9
ADAD	6	57.8 ± 10.6 (43–68)	3/3	4/1/1	9.5 ± 4.0	19.6 ± 8.7
<i>APP</i> _{swe}	3	56–68	0/3	2/0/1	9.3 ± 2.2	21.7 ± 1.3
<i>PS1</i> M146V	2	43–48	2/0	1/1/0	4–10	28
<i>PS1</i> E49	1	66	1/0	1/0/0	14	5
Ctrl	14	74.1 ± 12.0 (50–88)	5/9	14/0/0	—	7.3 ± 3.8

Abbreviations: sAD, sporadic Alzheimer's disease; ADAD, autosomal dominant Alzheimer's disease; *APP*_{swe}, Swedish *APP* mutation carrier; *PS1* M146V, *Presenilin 1* M146V mutation carrier; *PS1* E49, *Presenilin 1* exon 9 deletion; Ctrl, healthy age-matched controls; APOE, apolipoprotein E; 0/1/2, number of ϵ 4 allele.

NOTE. Data are shown as mean $\pm$ standard deviation (SD).

2.4. Saturation assays in the frontal cortex homogenates from sporadic AD

Postmortem frontal cortex brain tissue homogenates (100- μ g tissue) from patients with sAD were incubated with 0.01–250 nM 3 H-AZD2184, 3 H-PIB, 3 H-florbetaben, and methyl- 3 H-BTA-1 in $1 \times$ PBS buffer (pH 7.4) for 2 hours at room temperature. Nonspecific binding was determined in the presence of 1- μ M BTA-1. Fig. 6 shows the chemical structures of the ligands. Samples were run in triplicates, and the specific binding was expressed in pmol/g tissue. The dissociation constant (K_d) and maximum number of binding sites (B_{max}) were determined using nonlinear regression models in GraphPad Prism, version 6.0 (GraphPad Software, Inc., La Jolla, CA). The saturation data were fit to one-site and two-site binding model, followed by F-test for model selection. Scatchard plots were obtained using GraphPad Prism to display the saturation data.

2.5. Competitive binding studies in frontal cortical and striatum homogenates from sporadic AD and ADAD

Competitive binding assays comparing different amyloid tracers in sAD frontal cortex brains were carried out by incubating homogenates (100- μ g tissue) with 1.0-nM 3 H-PIB, 2.5-nM 3 H-florbetaben, 1.5-nM 3 H-AZD2184, or 2.0-nM methyl- 3 H-BTA-1 in the presence of unlabeled AZD2184, BTA-1, florbetaben and at concentrations ranging from 10^{-11} to 10^{-4} M. Competition binding assays comparing 3 H-PIB binding properties in sAD and ADAD (*APP^{swe}*, *PS1 M146V*, and *PS1 E49*) brain were carried out by incubating frontal cortex and striatum homogenates (100 μ g tissue) with 1.0-nM 3 H-PIB in the presence of BTA-1 and AZD2184 at concentrations ranging from 10^{-11} to 10^{-4} M. The affinity constant (K_i) and percentage of displacement were determined by using nonlinear regression one-site and two-site binding models derived from Cheng–Prusoff equation in GraphPad Prism, followed by F-test for model selection. The three-site binding of 3 H-PIB/AZD2184 competitive binding in the frontal cortex from ADAD was analyzed by additional fitting using two-site binding model in GraphPad Prism with binding data at AZD2184 concentration ranging from 10^{-9} to 10^{-4} M.

2.6. Regional 3 H-PIB and 3 H-AZD2184 binding in brain tissue homogenates from sporadic AD, ADAD, and healthy controls

Single concentration binding studies using 1.0-nM 3 H-PIB and 1.5-nM 3 H-AZD2184 were carried out by incubating homogenates (200 μ g tissue) from the frontal cortex and striatum of sAD and ADAD (*APP^{swe}*, *PS1 M146V*, *PS1 E49* mutation carriers) and control brains. Nonspecific binding was determined in the presence of 1- μ M BTA-1.

2.7. Interaction of resveratrol on amyloid tracer binding in the frontal cortical homogenates from patients with sporadic AD

To determine the influence of phenols on amyloid tracer binding, frontal cortex homogenates from three sAD were incubated with 1.5-nM 3 H-AZD2184, 2.5-nM 3 H-florbetaben, and 1.0-nM 3 H-PIB in the presence of resveratrol at concentrations of 10^{-11} to 10^{-4} M.

2.8. Statistical analysis

Data were analyzed using GraphPad Prism, version 6.0. Nonparametric Mann–Whitney test was used for comparisons between groups. All values are shown as means $\pm$ standard deviation (SD). Error bars in the figures represent standard error (SEM) values. Significant differences between groups were set at $*P < .05$, $**P < .01$, and $***P < .001$.

3. Results

3.1. Bielschowsky silver staining in sporadic AD and ADAD

Bielschowsky silver staining was performed in brain sections of *APP^{swe}*, *PS1 M146V*, and *PS1 E49* cases. ADAD showed a more intensive neuritic plaque staining in the layer I–III of frontal medial gyrus compared to sporadic AD cases (Fig. 1A–1D). The largest plaques were observed in the cortex of *PS1 E49* case reaching the size of 100 μ m in diameter. In *PS1 M146V* cases, neurofibrillary tangles and treads were less present, with the feature of heavy loaded silver-impregnated neuritic plaques indicating the presence of additional fibrillary pathology in the plaques. *APP^{swe}* and *PS1 M146V* cases showed intensive silver impregnation in the striatum; whereas for *PS1 E49* cases as well as sAD cases, there was an abundance of plaques in the striatum although the silver staining was weaker indicating their more diffuse nature (Fig. 1E–1H).

3.2. Comparison of the binding properties of different amyloid tracers in the frontal cortex and striatum of sporadic AD and ADAD

To characterize the binding sites of the amyloid tracers 3 H-AZD2184, 3 H-PIB, 3 H-florbetaben, and methyl- 3 H-BTA-1, saturation-binding studies using increasing concentrations (0.01–250 nM) of these tracers were performed in the frontal cortex homogenates from sAD patients. Fig. 2 illustrates the saturation curves and Scatchard plots. Two binding sites could be obtained for 3 H-AZD2184 (K_d : 6.5 nM, 50.4 nM), 3 H-PIB (K_d : 2.5 nM, 81.7 nM), and 3 H-florbetaben (K_d : 9.7 nM, 136 nM), respectively. Comparable binding affinity (K_d) for the high-affinity and low-affinity binding sites, and B_{max} for the high-affinity

Bielschowsky silver staining

Frontal cortex

Striatum

Fig. 1. Bielschowsky staining of the frontal medial gyrus, layers I–III (A–D) and striatum (E–H) in the brain slides from autosomal dominant Alzheimer's disease (*APP^{swe}*, *PS1 M146V*, *PS1 E49* mutation carrier) and sporadic Alzheimer's disease (sAD). The most intensive silver impregnation was obtained in cortical layers of ADAD and in the striatum of *APP^{swe}*, *PS1 M146V*, whereas striatum of *PS1 E49* has displayed weak staining indicating diffuse-like plaque structures. In the sporadic AD case, frontal cortex has expressed neuritic plaques less intensive than corresponding ADAD frontal cortex. In the striatum of sporadic AD case, diffuse plaques were dominant. In the section from striatum, the presence of fiber tracts (FT) indicates a proper anatomic level. Bar = 200 μ m.

site was obtained for three amyloid tracers. No definite binding sites could be detected with the tracer methyl- 3 H-BTA-1 due to high percentage of nonspecific binding.

To further characterize and compare the binding properties of the amyloid tracers, competition studies were performed in the frontal cortex homogenates from sAD cases with 3 H-AZD2184, 3 H-PIB, 3 H-florbetaben, and methyl- 3 H-BTA-1 in the presence of increasing concentrations of unlabeled BTA-1, AZD2184, and florbetaben (10^{-11} to 10^{-4} M), respectively. Analysis of the competition results demonstrated the presence of a similar high-affinity site and a varying low-affinity site of 3 H-AZD2184, 3 H-PIB, and 3 H-florbetaben in the sAD frontal cortex (Fig. 3).

3 H-PIB showed two binding sites for unlabeled AZD2184 (K_i : 0.1 nM, 125 nM), BTA-1 (K_i : 0.9 nM, 83 nM), and florbetaben (K_i : 0.1 nM, 190 nM) in the sAD frontal cortex.

3 H-AZD2184 demonstrated a wide range of low-affinity binding sites with unlabeled AZD2184 (K_i : 0.1 nM, 7 nM), BTA-1 (K_i : 0.1 nM, 53 nM), and florbetaben (K_i : 0.2 nM, 325 nM). For 3 H-florbetaben, two sites with comparable affinities were obtained with AZD2184 (K_i : 0.5 nM, 24 nM), BTA-1 (K_i : 0.1 nM, 45 nM), and florbetaben (K_i : 0.1 nM, 32 nM), respectively. Methyl- 3 H-BTA-1 binding contained high percentage of nonspecific component, and one binding site was estimated for florbetaben ($\approx$ 20 nM), AZD2184 ($\approx$ 200 nM), and BTA-1 ($\approx$ 1000 nM; Fig. 3).

To investigate the difference between amyloid binding sites in ADAD and sAD brain, 3 H-PIB competitive binding assays were performed using unlabeled AZD2184 or BTA-1 in the frontal cortex and striatum from six ADAD patients (including three *APP^{swe}*, two *PS1 M146V*, and one *PS1 E49* mutation carriers). In the frontal cortex from ADAD,

sAD Frontal cortex

Fig. 2. Saturation binding of different amyloid tracers in the frontal cortices from sporadic Alzheimer's disease. (A, B) ^3H -AZD2184 (0.1–150 nM), (C, D) ^3H -PIB (0.1–250 nM), (E, F) ^3H -florbetaben (0.1–200 nM) saturation in frontal cortices from sporadic Alzheimer's disease indicated a similar high-affinity and a low-affinity binding site for ^3H -AZD2184 ($K_{d1} = 6.5 \text{ nM}$, $B_{\max1} = 2210 \text{ pmol/g tissue}$; $K_{d2} = 50.4 \text{ nM}$, $B_{\max2} = 5897 \text{ pmol/g tissue}$), ^3H -PIB ($K_{d1} = 2.5 \text{ nM}$, $B_{\max1} = 1677 \text{ pmol/g tissue}$; $K_{d2} = 81.7 \text{ nM}$, $B_{\max2} = 8120 \text{ pmol/g tissue}$), ^3H -florbetaben ($K_{d1} = 9.7 \text{ nM}$, $B_{\max1} = 1597 \text{ pmol/g tissue}$; $K_{d2} = 136 \text{ nM}$, $B_{\max2} = 12,209 \text{ pmol/g tissue}$). Binding data were analyzed using GraphPrism non-linear regression fitting. Abbreviations: sAD, sporadic Alzheimer's disease; ^3H -PIB, ^3H -Pittsburgh compound B.

three binding sites were demonstrated using AZD2184 (K_i : 0.5 nM, 33 nM, 327 nM), whereas two binding sites with unlabeled BTA-1 (K_i : 1.3 nM, 80 nM; Fig. 4A). In the striatum of ADAD, two binding sites were detected using AZD2184

(K_i : 0.6 nM, 95 nM) and BTA-1 (K_i : 0.8 nM, 37 nM). The ^3H -PIB/BTA-1 binding affinity was somewhat higher in the striatum of ADAD cases compared to that of sAD (K_i : 4.8 nM, 105 nM; Fig. 4B).

Fig. 3. Competitive binding of different amyloid tracers in the frontal cortex from sporadic Alzheimer's disease showing multiples binding sites. (A) ^3H -AZD2184 (1.5 nM), (B) ^3H -PIB (1.0 nM), (C) ^3H -florbetaben (2.0 nM), and (D) ^3H -Methyl-BTA-1 (2.5 nM) binding displaced with unlabeled AZD2184 (blue), BTA-1 (red), florbetaben (black) at 10^{-12} M– 10^{-4} M in the frontal cortex from sporadic Alzheimer's disease ($n = 3$ –6). Data are presented as means $\pm$ standard error of the mean (SEM). Abbreviations: sAD, sporadic Alzheimer's disease; ^3H -PIB, ^3H -Pittsburgh compound B; ^3H -Me-BTA-1, ^3H -Methyl-BTA-1.

3.3. Regional amyloid tracer binding in autosomal dominant and sporadic Alzheimer's disease

The binding of a single concentration of ^3H -PIB (1.0 nM) and ^3H -AZD2184 (1.5 nM) was measured in the homogenates from the frontal cortex and striatum of six ADAD cases (including three *APP*^{swe}, two *PS1* M146V, and one *PS1* E49 mutation carriers), 13 sAD cases, and 14 controls respectively. Although the three ADAD mutations showed different properties of plaque pathology (Fig. 1A–1H), the ADAD cases were analyzed together due to few cases

from each mutation. A significant higher number of ^3H -PIB and ^3H -AZD2184 binding sites were observed in the frontal cortex of both ADAD and sAD cases compared to healthy controls (Table 2). A significant higher ^3H -PIB as well as the ^3H -AZD2184 binding was measured in the striatum of ADAD cases compared to control cases, whereas no significant difference in binding was observed between sAD and controls (Table 2). Of the ADAD cases, both cases with *APP*^{swe} and *PS1* M146V encoded mutations showed heavy plaque load in the striatum (Fig. 1E–1H).

Fig. 4. Competitive binding of amyloid tracers in the frontal cortices and striatum from sporadic and autosomal dominant Alzheimer's disease. (A) ^3H -PIB (1.0 nM) displacement with 10^{-12} M– 10^{-4} M AZD2184 and BTA-1 in the frontal cortices from autosomal dominant Alzheimer's disease (*APP^{swe}*, *PS1 M146V* and *PS1 E49* mutation carriers). (B) ^3H -PIB (1.0 nM) displacement with 10^{-12} M– 10^{-4} M AZD2184 and BTA-1 in the striatum from sporadic and autosomal dominant Alzheimer's disease. Binding data were analyzed using GraphPrism non-linear regression fitting. Data are presented as means $\pm$ standard error of the mean (SEM). Abbreviations: sAD, sporadic Alzheimer's disease; ADAD, autosomal dominant Alzheimer's disease; ^3H -PIB, ^3H -Pittsburgh compound B.

3.4. Interaction of resveratrol with amyloid tracers binding in the frontal cortex of sporadic Alzheimer's disease

To investigate the possible interactive mechanisms between resveratrol and A β tracers' binding sites in sAD brain, competitive binding assays were performed using resveratrol (10^{-11} to 10^{-4} M) with ^3H -AZD2184 (1.5 nM), ^3H -PIB (1.0 nM), and ^3H -florbetaben (2.0 nM), respectively, in homogenates prepared from the frontal cortex of three sAD cases. Analysis of the resveratrol competition binding studies demonstrated an interaction with a high-affinity binding site for ^3H -AZD2184 ($K_i \approx 5$ nM), while for ^3H -florbetaben to a low affinity site ($K_i \approx 238$ nM) and a very low affinity site for ^3H -PIB ($K_i \approx 1900$ nM; Fig. 5).

4. Discussion

Amyloid PET imaging has advanced our understanding of the early presymptomatic deposition of fibrillar A β in patients with ADAD and sAD [14,18,19]. There is a need to understand the interaction of A β ligands on the different binding sites on the A β fibril β -sheet and to clarify their biological roles and possible technical confounders [20–22]. In this study, we examined the *in vitro* properties of A β tracers and studied how different chemical structures (Fig. 6) could influence the interaction of different A β conformation characteristics in ADAD and sAD cortical and striatal brain tissue, providing a link between binding and A β neuropathology.

Clinical studies using different A β PET tracers have shown comparable results in detecting the presence of

Table 2

Regional distribution of amyloid tracer ^3H -PIB (1.0 nM) and ^3H -AZD2184 (1.5 nM) binding in brain from sporadic, autosomal dominant Alzheimer's disease and healthy controls

Brain region	Ligand	ADAD	sAD	Control
Frontal cortex	^3H -PIB (pmol/g tissue)	212.2 $\pm$ 46.5** (n = 5)	219.0 $\pm$ 66.6*** (n = 8)	15.4 $\pm$ 20.5 (n = 14)
	^3H -AZD2184 (pmol/g tissue)	409.9 $\pm$ 171.6** (n = 5)	273.4 $\pm$ 103.7*** (n = 8)	19.9 $\pm$ 31.0 (n = 14)
Striatum	^3H -PIB (pmol/g tissue)	133.7 $\pm$ 43.5*** (n = 6)	53.7 $\pm$ 68.9# (n = 7)	5.6 $\pm$ 8.3 (n = 8)
	^3H -AZD2184 (pmol/g tissue)	237.5 $\pm$ 85.2* (n = 6)	179.7 $\pm$ 172.6 (n = 7)	27.7 $\pm$ 32.6 (n = 6)

Abbreviations: sAD, sporadic Alzheimer's disease; ADAD, autosomal dominant Alzheimer's disease; ^3H -PIB, ^3H -Pittsburgh compound B.

NOTE. Data are presented as means $\pm$ standard deviation (SD).

NOTE. Significant differences compared to control group (Mann–Whitney *U* test) are indicated by * $P < .05$, ** $P < .01$, *** $P < .001$. Significant difference between ADAD and sAD group is indicated by # $P < .05$.

Fig. 5. Effect of resveratrol (10^{-11} M– 10^{-4} M) on the amyloid tracers binding in the frontal cortices from sporadic Alzheimer's disease. (A) ^3H -AZD2184 (1.5 nM), (B) ^3H -PIB (1.0 nM), and (C) ^3H -florbetaben (2.0 nM), in frontal cortex brain homogenate from sporadic Alzheimer's disease ($n = 3$). The interaction with resveratrol on ^3H -AZD2184 was estimated to $K_i \approx 5$ nM, ^3H -florbetaben $K_i \approx 238$ nM and ^3H -PIB $K_i \approx 1900$ nM. ^3H -PIB = ^3H -Pittsburgh compound B; Binding data were analyzed using GraphPrism nonlinear regression fitting. Data are presented as means $\pm$ standard error of the mean (SEM).

fibrillar A β plaques in brain from sAD and mild cognitive impairment using ^{11}C -PIB [23], ^{18}F -florbetaben [24], ^{18}F -florbetapir, ^{18}F -flutemetamol [25,26], and ^{18}F -NAV4694 [27]. Head-to-head comparison of ^{11}C -PIB and ^{18}F -AZD4694 has shown strong correlation in binding [27] as well as between the three tracers ^{11}C -PIB, ^{18}F -florbetapir, and ^{18}F -flutemetamol [28]. In A β microPET in transgenic AD rat brain, excessive NAV4694 (an AZD2184 analog) blocked only 10%–25% ^{11}C -PIB binding, suggesting that these two tracers may bind to different sites or forms of A β [29]. Such competitive experiment is highly informative but not available in AD patients.

^3H -PIB binding correlates with amyloid plaque loads in sAD brain [30,31], including neuritic plaques, cerebral angiopathy (CAA), diffuse plaques, but not amorphous amyloid plaques in AD brain [32]. ^{18}F -flutemetamol [26], ^{18}F -florbetapir [33], and ^{18}F -florbetaben PET have shown high sensitivity and specificity for the detection of histopathology-confirmed neuritic A β plaques. A subfraction of A β has been found containing high-affinity ^3H -PIB site in sAD brain [34]. Recent studies reported that Thal phase but not CERAD score correlates with PIB SUV from antemortem imaging, suggesting that diffuse plaques may contribute to the PIB signal in PET imaging [32].

From the chemical structure perspective, Thioflavine T-derived tracer ^3H -AZD2184, ^3H -PIB, methyl- ^3H -BTA-1, and stilbene-derived tracer ^3H -florbetaben all share a planar structure. In addition, florbetaben contains a more flexible polyethylene glycol (PEG) chain, which maintain lipophilicity and neutrality may influence the compounds engagement with β -sheet structure [35]. All studied A β tracers (^3H -florbetaben, ^3H -AZD2184, ^3H -PIB) except ^3H -Me-BTA-1 showed both a high-affinity and a low-affinity binding site

in AD brain tissue, in agreement with earlier studies [7]. AZD2184 showed higher affinity of binding sites in the frontal cortex of sAD and a third binding site in the frontal cortex of ADAD, supporting multiple binding site model on fibrillar A β in brain. Earlier biophysical modeling studies have identified six possible binding sites for A β tracers PIB, BTA-1, and Thioflavine T [3,4]. Florbetaben, florbetapir, and PIB that of different scaffolds share a common binding mode to surface groove, whereas the particular binding position and affinity might vary between different amyloid tracers and morphologies of A β fibrils [5] which is in line with present study. When the binding profile of AZD2184 and thioflavine was studied by molecular docking and molecular dynamic simulation, four binding sites were observed on the A β fibril where three with the fibril and one on the two sides of the Met35 residue on the surface [35]. The binding affinity of AZD2184 was found to be higher than thioflavine due to electrostatic interaction and spatial restriction [35]. Additionally, factors such as lipophilicity, pKa, and the overall tracer structure may influence the tracer binding properties, and the low nonspecific binding of ^3H -AZD2184 has been attributed partly to its low lipophilicity (^3H -AZD2184 < ^3H -PIB < ^3H -florbetaben < Methyl- ^3H -BTA-1) [36,37].

We found that resveratrol interacted with the rank order of AZD2184 > florbetaben > PIB. Several possible mechanisms have been proposed regarding resveratrol-A β interaction, including specific conformation or noncovalent interaction with β -sheet structure [38] or with A β sequence such as the A β_{17-21} aromatic phenylalanine residue. Resveratrol are currently under clinical trial and phase II clinical trial in AD patients (NCT01504854). Interestingly enough resveratrol is a phenol with stilbene structure. Resveratrol has been shown to inhibit synthetic A β aggregation and

Fig. 6. Chemical structures of different amyloid positron emission tomography (PET) tracers and the polyphenol resveratrol.

toxicity *in vitro* [39] and detected fluorescence of A β plaques similarly as Thioflavin T in postmortem AD brain slides [40,41]. The indication of direct binding of resveratrol to A β has raised an interest in developing resveratrol derivatives as potential A β PET tracers [42], luminescent conjugated polymers as well as AD treatment agents. In a recent study, administration of resveratrol (200 mg/day) for 26 weeks in healthy, overweight older adult showed an improvement in memory performance as well as improved glucose metabolism [43].

Recent Alzheimer Disease Neuroimaging Initiative study and Dominantly Inherited Alzheimer Network have identified higher total A β load and less variety in comorbidity in ADAD brain [44]. We examined the properties of different A β tracers in brain tissue from mutation carriers with different characteristic AD pathology [45–49] and compared with that in sAD. We demonstrated that the number of striatal ^3H -PIB and ^3H -AZD2184 binding sites in ADAD was higher compared to sAD and did not differ in the frontal cortex between two AD groups. This observation is in agreement with an earlier report [10], which also demonstrated a higher ^{11}C -PIB retention *in vivo* in the striatum of *PS1 E49* mutation carriers compared to sAD. The lower intensity of silver impregnation of the plaques (mainly diffuse) in the striatum of *PS1 E49* mutation case underlines the unique biochemical feature of the fibrillation of that mutation. *PS1 E49* muta-

tion carriers are known for their characteristic pathology of “cotton wool” plaques and CAA in the frontal cortex and widespread small diffuse A β plaques devoid of dystrophic neurites in the striatum [48]. The finding suggests that A β tracers can detect diffuse plaques. The arctic *APP* mutation carriers are lacking the β -sheet formation and show low ^{11}C -PIB brain retention [13]. A very high plaque load is presented in the *APP^{swe}* and *PS1 M146V* brain tissue. Despite that A β tracers have been suggested to bind to late fibrillar forms, the findings in the present study suggest that intensity of the binding thus not only depends on the morphological feature of the silver impregnation but probably rather more on the specific biochemical properties of plaques especially in the particular mutation, and the sensitivity of the ligand binding even to the mutation-specific earlier/diffuse plaque.

There are some limitations of this study. First, the fact that the availability of autopsy brain material is rare from these very unusual mutations which does not allow large-scale studies. Second, *in vitro* solid-state nuclear magnetic resonance studies suggest that the molecular structures of A β fibrils vary in different sAD brains, which may contribute to the observed variation [50]. For quality control of the present study, the experiments were therefore repeated independently more than five times and the binding curve analysis was evaluated independently and discussed between three of the authors. Third, the variance

in the competitive binding studies with resveratrol and different amyloid tracer was rather large as resveratrol is not specific for A β fibrils.

Understanding the binding mode of different amyloid tracers to brain A β aggregates may facilitate the development of new early biomarkers as well as designing new drug targets for AD therapies. Future study regarding the pathologic properties of the arctic and E293 Δ mutations brains including studies of regional tau deposition and astrocytes activation will be insightful. New strategies for developing new A β tracers that can detect smaller forms of A β oligomeric forms as well protofibril are of great interest and should be tested in *in silico* modeling as well as in ADAD especially arctic mutation brain tissue and further also be explored by *in vivo* imaging in ADAD subjects at risk as well as sAD patients. Competitive binding using different amyloid compounds *in vivo* in AD patients would be informative but might be difficult due to the high concentrations needed of the tracers. In addition, it will be of clinical relevance to study how differences in properties of amyloid tracers could discriminate between rapidly compared to slowly progressive AD cases and also might detect variants of late-onset sAD with divergent A β pathology.

To conclude, the assessed A β tracers in the present study showed multiple binding sites and different binding properties in ADAD in comparison to sAD brains. AZD2184 detected a third A β binding site in the ADAD frontal cortex. High binding of the A β tracers in ADAD striatal tissue suggests conformational differences of amyloid aggregates in ADAD compared to sAD, confirming earlier *in vivo* PET observations in the cortex and striatum of sAD and ADAD patients. The anti-amyloid phenol compound resveratrol interacted in nanomolar range with AZD2184 but showed less affinity to florbetaben followed by PIB. Studies of the target similarity, differences, and selectivity properties between different amyloid tracers, and *in vivo*/postmortem validation will facilitate the development of new imaging and therapeutic agents action on specific A β conformational aggregates.

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RESEARCH IN CONTEXT

1. Systematic review: Literature search using autosomal dominant Alzheimer's disease (ADAD), sporadic Alzheimer's disease (sAD), amyloid- β imaging. *In silico* studies indicate that different amyloid tracers might bind to different site on the amyloid beta sheets. The aim of the study was to characterize the binding properties of amyloid tracers in ADAD brain in comparison to sAD.
2. Interpretation: Amyloid tracers such as ^3H -PIB, ^3H -florbetaben, ^3H -AZD2184 and BTA-1 share a high-affinity binding site and a large variation in affinity of low-affinity binding site in sAD cases. A high-affinity binding site is observed in ADAD striatal tissue and a third binding sites for ^3H -AZD2184 in the frontal cortex compared to sAD. The polyphenol resveratrol showed highest interaction with AZD2184 binding (nanomolar range). The findings suggest that the amyloid tracer binding depends both on the tracer structure as well as the amyloid- β fibrils conformations.
3. Future directions: Further studies regarding amyloid- β polymorphism and differences between ADAD and sAD.

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