EVALUATION OF (^{18}F)-FEDAA AS A PET-RADIOTRACER FOR THE IMAGING OF ATHEROSCLEROSIS IN MAN AND A RABBIT MODEL

J.L. BIRD [1], K.C. PROBST [1], D. IZQUIERDO [1], A.P. DAVENPORT [2] and E.A. WARBURTON [1]

[1]BHF Imaging Group, Dept. of Neurosciences, University of Cambridge, Cambridge, United Kingdom; [2]Dept. of Medicine, University of Cambridge, Cambridge, United Kingdom

Introduction: Atherosclerotic plaques are ubiquitous in man and their rupture precipitates most heart attacks and strokes. Plaque instability culminating in rupture correlates not with plaque size or the degree of luminal stenosis, but with the inflammatory activity within the plaque. A consistent feature is an elevated number of activated macrophages. Previously, we have demonstrated the potential of positron emission tomography (PET) to image inflammatory activity in both carotid atheroma in humans (1) and aortic atheroma in a rabbit model using [^{18}F]- fluorodeoxyglucose (FDG) (2). The 18kDa peripheral benzodiazepine receptor (PBR) is a unique class of diazepine receptor which is functionally distinct from the central diazepine receptor and performs a number of context-dependent functions including cholesterol transport. It is expressed in a variety of tissues and cell types, including immune cells such as the macrophage as well as kidney and heart. *In vivo* imaging of the PBR is possible by (PET) using appropriate radioligands and has clinical potential.

Experimental: Here, we evaluate [^{18}F]-FEDAA1106; *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[^{18}F]-fluoroethyloxy-5-methoxybenzyl)acetamide (3) as a radioligand for PBR expression by binding assay and immunohistochemistry. Our aim is to characterise the binding of [^{18}F]-FEDAA1106 to PBR as it is potentially a more specific marker of macrophage activity than FDG.

Results and Discussion: We have demonstrated that [^{18}F]-FEDAA binds specifically to a number of human and rabbit tissues known to express PBR. Analysis of [^{18}F]-FEDAA binding in human carotid and rabbit aortic atheroma *ex vivo* indicates that this radioligand preferentially binds to sites within the plaque (Fig. 1); these sites correlate with sites of PBR expression and macrophage presence (CD68).

Fig. 1. [^{18}F]-FEDAA1106 (0.5 nM) binding to rabbit (A, B) and human (C, D) atheroma in the absence (A, C) and presence (B, D) of non-radioactive FEDAA (10 μM).

Conclusion: Confirmation of the specificity of [^{18}F]-FEDAA1106 for PBR expressed in activated macrophages in the atherosclerotic plaque is a necessary prequel to evaluating the efficacy of this PET radioligand in a rabbit model of atheroma. The successful conclusion of these experiments will generate a powerful clinical tool for determining atheroma instability in man.

Acknowledgement: British Heart Foundation.

References: [1] Rudd JHF *et al* (2002) *Circulation* 105; 2708-2711. [2] Izquierdo D et al (2006) *Molecular Imaging* 5; 310 (Abs. 430). [3] Zhang MR. *et al* (2004) *J Med Chem* 47; 2228-2235.

Keywords: Atherosclerosis, PET-Imaging, PBR

(^{11}C)MES-IMPYS AS POTENTIAL RADIOLIGANDS FOR BETA-AMYLOID

L. CAI, J.-S. LIOW, J. CUEVAS, C. BAETA, J. HONG, S.S. ZOGHBI, R.B. INNIS and V.W. PIKE

Molecular Imaging Branch, National Institute of Mental Health, Bethesda, MD, USA

Introduction: IMPY (4-(6-iodoimidazo[1,2-*a*]pyridin-2-yl)-*N*,*N*-dimethylaniline) derivatives, previously evaluated as prospective radioligands for A-beta amyloid aggregates in Alzheimer's Disease (AD), have shown rapid and high uptake of radioactivity into brain and quick washout. However, there is residual radioactivity, most likely radiometabolite. With a view to evaluate more derivatives of IMPY, we used an "isoelectronic effect" in the design of IMPY analogs. Here we report MeS-IMPY, its labelling with ^{11}C in either of two positions, as in **1-S** or **1-N** (Fig. 1), and kinetic study of these radioligands with PET.

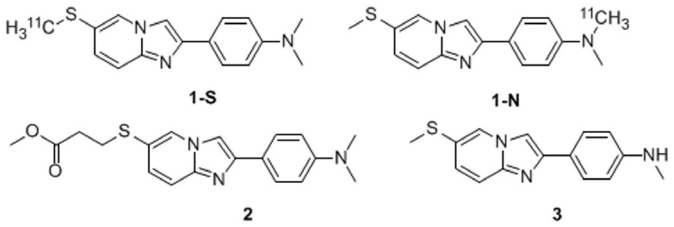


Fig. 1. Key chemical structures.

Experimental: The radiosynthesis is shown in Fig. 2.

Results and Discussion: MeS-IMPY has high affinity (K_i = 7.9 ± 2.7 nM) for AD brain homogenates *in vitro* (*c.f.* K_D = 7.2 nM for PIB). The uptake of radioactivity into rat brain after *i.v.* injection of **1-S** was high (200% SUV at 0.5–1.2 min, n = 4) but less than that of the currently established radioligand, [^{11}C]PIB (300% SUV at 0.5 min; n = 1). However, the ratio of radioactivity at its maximum to that at 60 min was ~ 18 (*c.f.* ~ 9 for [^{11}C]PIB). In monkey, **1-S** gave a corresponding ratio of 5–10, higher than ~ 5 for [^{11}C]PIB. When **1-N** was used in the PET imaging experiment of rat, the ratio of maximum radioactivity to that at 60 min was only ~ 6, indicating radiometabolite entrapment. ATLAS-PET imaging of **1-S** in 20 mo old TgCRND8 mice revealed a higher ratio of cortical to cerebellar radioactivity than in wild type mice (Fig. 3).

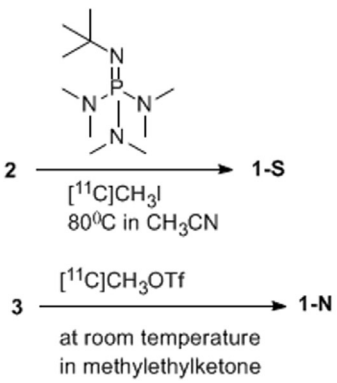


Fig. 2. Radiochemical synthesis.

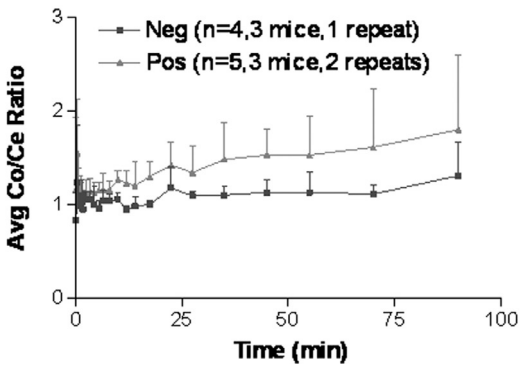


Fig. 3. Cortical to cerebellum radioactivity ratio after injection of **S-1** into Tg and wild type control mice.

Conclusion: The brain kinetics of **1-S** (but not **1-N**) compare favorably with those of [^{11}C]PIB. PET brain imaging with **1-S** in Tg mice suggests some specific binding to A-beta amyloid. Further investigation of **1-S** in AD patients is in progress.

Acknowledgement: This research was supported by the Intramural Research Program of the NIH, NIMH.

Keywords: Radiotracer, PET, Amyloid, Alzheimer, Transgenic Mice

^{18}F-LABELED STILBENES FOR IMAGING β-AMYLOID PLAQUES IN THE BRAIN

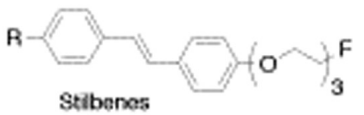
W. ZHANG, M. KUNG, S. OYA, C. HOU and H. KUNG

Radiology, University of Pennsylvania, Philadelphia, PA, USA

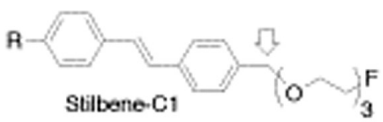
Introduction: One of the hallmarks of Alzheimer's disease (AD) is the accumulation of β-amyloid plaques (Aβ aggregates) in the brain. It has been considered as one of the pathological factors associated with AD. Thus, PET imaging agents, targeting Aβ plaques, will be useful for diagnosis and monitoring disease progression. To enhance in vivo stability and evaluate structure activity relationship of the fluoro-pegylated derivatives, we compared a series of 18F labeled PEG derivatives of stilbenes targeting Aβ plaques. In these series of compounds, 18F is linked to the stilbenes and C1 through a triethylene glycol chain. The glycol group lowers the molecule's lipophilicity and provides a convenient attachment point for 18F labeled FPEG.

Experimental: The syntheses of fluoro-PEG derivatives containing either N-monomethyl group of stilbene and stilbene-C1 were achieved. In vitro binding studies using postmortem AD brain homogenates and [^{125}I]IMPY showed that the N-monomethyl and N,N-dimethyl derivatives of stilbene and stilbene-C1 derivatives displayed excellent binding affinities to Aβ aggregates. 18F labeling was successfully performed by a substitution of the mesylate group with [^{18}F]fluoride in good radiochemical yields (EOS >20%). The specific activity of the 18F labeled ligands at the end of synthesis reached 1000 Ci/mmol.

Results and Discussion: In vivo biodistribution of these novel 18F ligands, both N-monomethyl and N,N-dimethyl derivatives, tested in normal mice exhibited excellent brain penetrations and rapid washouts after iv injections. Autoradiography of postmortem AD brain sections confirmed the specific signal due to the presence of Aβ plaques. Extension of FPEG attachment to the stilbene by one more carbon retained the desired Aβ plaque binding properties.



Stilbenes



Stilbene-C1

	K_i (nM)	
R	Stilbenes	Stilbene-C1
-NH_2	25	34
-NHMe	6.7	4.8
-NMe_2	2.1	2.0

Conclusion: In conclusion, the preliminary results strongly suggest that the fluorinated PEG derivatives of stilbene and stilbene-C1 derivatives are excellent candidates as Aβ plaque imaging agents for studying the accumulation of Aβ aggregates in AD patients.

Acknowledgement: This work was supported by a grant awarded from the National Institutes of Health (R01-AG-022559, H.F.K.).

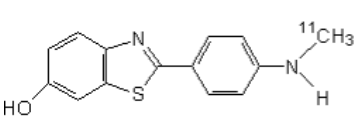
Keywords: Brain Imaging, Alzheimer's Disease, In Vitro Binding, F-18 Radiochemistry, Biodistribution

2-(3'-([11]C)METHYLAMINO-4'-AMINOPHENYL)-1,3-BENZOTHIAZOLE AS POTENTIAL AMYLOID IMAGING AGENT

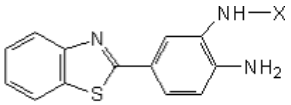
K. SERDONS[1], C. LANDELOOS[1], C. TERWINGHE[2], P. VERMAELEN[2], K. VAN LAERE[2], H. KUNG[3], G. BORMANS[1] and A. VERBRUGGEN[1]

[1]Laboratory of Radiopharmacy, K.U. Leuven, Leuven, Belgium; [2]Department of Nuclear Medicine, U.Z. Gasthuisberg, Leuven, Belgium; [3]Department of Radiology, University of Pennsylvania, Philadelphia, USA

Introduction: Pittsburgh Compound-B (6-hydroxy-2-(4'-N-[^{11}C]methylaminophenyl)-1,3-benzothiazole, [^{11}C]6-OH-BTA-1, PIB, **1**) is currently being clinically evaluated for *in vivo* diagnosis of Alzheimer's disease (AD). We have now developed and evaluated a carbon-11 labelled PIB derivative with two amino substituents on the 2-phenyl ring (**2**) as a new potential *in vivo* positron emission tomography (PET) tracer for Alzheimer's disease.



1



2: X = $^{11}CH_3$

3: X = CH_3

Experimental: The affinity (Ki) of the 'cold' compound 2-(3'-methylamino-4'-aminophenyl)-1,3-benzothiazole (**3**) for *post mortem* brain homogenates of AD patients containing amyloid was determined following a described procedure (Kung MP 2004). Radiolabelling to prepare ^{11}C-labelled **2** was realized using [^{11}C]methyl triflate (3 min, 70°C). The log P value of RP-HPLC purified **2** was determined in octanol/0.025M phosphate buffer pH 7.4. Biodistribution of **1** and **2** was studied *ex vivo* at 2 and 60 min p.i. in normal mice and *in vivo* in a normal rat with a μPET camera.

Results and Discussion: Affinity of **3** for *post mortem* human AD brain homogenates was 6.0 nM. The log P value of **2** was 1.5. In normal mice, brain uptake of **2** was high at 2 min p.i. (3.2% ID, 10.5% ID/g) and wash-out from normal brain was rapid (at 60 min p.i.: 0.08% ID, 0.27% ID/g). The μPET study in a rat confirmed a high brain uptake and fast wash-out of this compound.

Conclusion: The new 2-(3'-methylamino-4'-aminophenyl)-1,3-benzothiazole shows good affinity for human brain homogenates containing amyloid. The Ki-value is in the same order as that of **1** which is 2.8 nM. The biodistribution and μPET study show favorable characteristics. The new carbon-11 labelled phenylbenzothiazole **2** has a higher brain uptake than PIB (**1** at 2 min p.i.: 1.08% ID, 3.6% ID/g). Brain wash-out (% ID cerebrum 2 min/% ID cerebrum 60 min) of **2** in normal mice is 6 times faster than that of PIB. The excellent characteristics of the new ^{11}C-labelled compound suggest that it is a promising candidate for *in vivo* visualization of amyloid.

Acknowledgement: This study was funded in part by the EC – FP6-project DiMi, LSHB-CT-2005-512146.

Keywords: Amyloid Plaque Imaging, Alzheimer's Disease, Carbon-11

SYNTHESIS AND EVALUATION OF ([18]F)FBTA, A POTENTIAL AMYLOID IMAGING AGENT

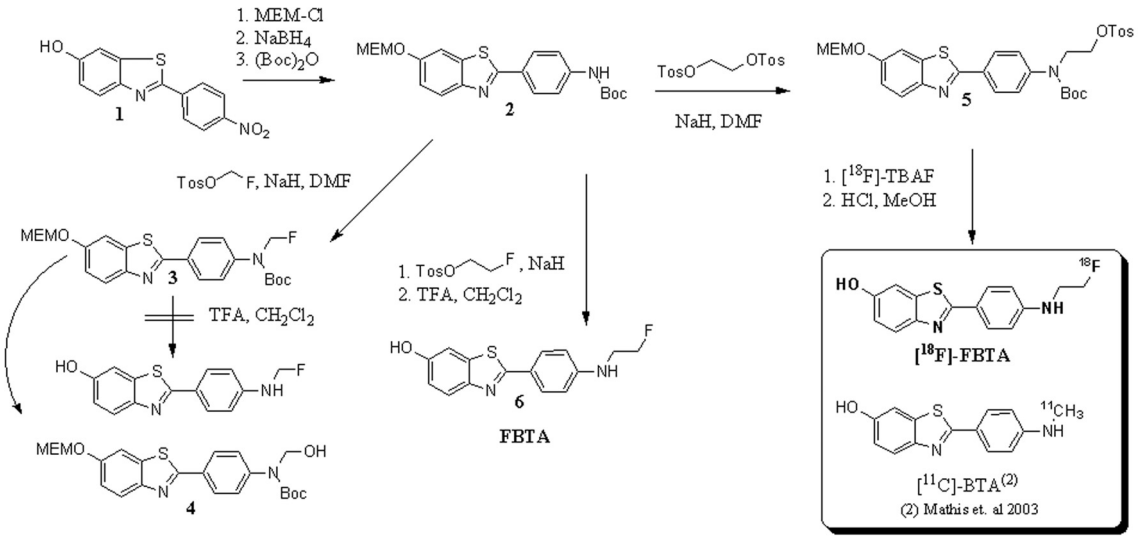
U. BERNDT [1,2], C. STANETTY [1], M. BERGER [3], H. KVATERNIK [2], T. WOLF [2], C. KUNTNER [2], T. WANEK [2], P. ANGELBERGER [2] and C. NOE [1]

[1]Dept. of Medicinal Chemistry, Faculty of Life Sciences, University of Vienna, Vienna, Austria; [2]Dept. of Radiopharmaceuticals, Austrian Research Centers GmbH – ARC, Seibersdorf, Austria; [3]Brain Research Institute, University of Vienna, Vienna, Austria

Introduction: β-Amyloid(Aβ) plaques and neurofibrillary tangles detected in post mortem brain are the defining characteristics of Alzheimer's disease (AD). In vivo imaging of Aβ plaques could be very useful for early diagnosis and therapy monitoring of AD. A 11C-labelled benzothiazole-aniline, [11C]BTA, is the first Aβ PET-tracer used in AD patients with promising results (1). Here we report the radiosynthesis and evaluation of [18F]FBTA, a new [18F]analog of [11C]BTA.

Experimental: Based on literature (2) 2-(4'-nitrophenyl)-6-hydroxybenzothiazole **1**, was synthesized. The hydroxy group was protected as methoxyethoxymethyl ether. Reduction of the nitro group led to the aniline. To avoid dialkylation in the next step the primary amine was protected with a Boc group to give **2**. 2-Fluoroethyl tosylate was used for fluoroethylation of the carbamino group. Deprotection of the amino and hydroxy group was carried out in one step to give 2-(4'-(2-fluoroethylamino)phenyl)-6-hydroxybenzothiazole **6**, abbreviated as FBTA. To avoid a two-step reaction with [18F]fluoroethyl tosylate another reaction path was chosen for the hot synthesis. **2** was alkylated with ethylene bis-tosylate to give the tosylethyl derivative **5**. This precursor was transformed into the [18F]fluoroethyl compound via nucleophilic substitution with [18F]TBAF and finally deprotected with HCl/MeOH to yield [18F]FBTA, which was isolated by semipreparative HPLC.

Results and Discussion: The structure of FBTA **6** was confirmed by 1H-NMR and ESI-MS. [18F]FBTA was obtained in 20 ± 6 (n=8) % radiochemical yield and 1.4 mCi/nmol mean specific activity, as analyzed by HPLC calibrated with FBTA **6** and shown to be stable in vitro.The initial goal of our synthesis was the corresponding fluoromethyl compound **3** as closest structural analog of [11C]BTA. **3** was successfully synthesized but it turned out to be unstable and degraded to **4** on storage and under deprotection.



Conclusion: Radiosynthesis of a new [18F]analog of [11C]BTA was successfully accomplished. Evaluation of [18F]FBTA by autoradiography of human AD brain sections and microPET in a transgenic mouse model is under investigation.

References: [1] H. Engler et al, Brain 2006; 129: 2856-66. [2] C.A. Mathis et al, J. Med. Chem. 2003; 46: 2740-54.

Keywords: [18F]FBTA, PET, Alzheimer's Disease, β-Amyloid, [11C]BTA

SYNTHESIS AND EVALUATION OF (^{18}F)PIB ANALOGS AS Aβ PLAQUE PET IMAGING AGENTS

N.S. MASON [1], W.E. KLUNK [2], M. DEBNATH [2], N. FLATT [1], G. HUANG [1], L. SHAO [2] and C.A. MATHIS [1]

[1]Radiology, PET Facility, University of Pittsburgh, Pittsburgh, PA, USA; [2]Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

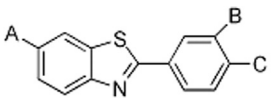
Introduction: We have been interested in the development of PET radioligands for use in non-invasive imaging of Alzheimer's disease (AD). These efforts have led to the development of neutral, lipophilic derivatives of thioflavin-T as potential PET radioligands for the detection and quantification of amyloid-β (Aβ) plaque deposits that are a hallmark of AD. One of these thioflavin-T derivatives, {N-methyl-[C-11]}2-(4'-methylamino-phenyl)-6-hydroxybenzothiazole ([C-11]PIB), has demonstrated the ability to detect Aβ plaque deposits *in vivo* in humans and has served as our lead compound in this series [1–3].

Experimental: We have used PIB as a starting point in our continued efforts to develop [F-18]-labeled analogs suitable for Aβ plaque imaging studies. These efforts led to the synthesis and evaluation of several analogs of PIB (**2-11**). These compounds have been evaluated using *in vitro* measures (log P_{C18}, and Ki), *ex vivo* measures from rodents (normal brain uptake and clearance), and *in vivo* measures in baboons (regional normal brain uptake and clearance) to determine their suitability as *in vivo* PET imaging candidates.

Results and Discussion: Facile radiolabeling, excellent *in vitro* and *ex vivo* properties, and encouraging non-human primate imaging results led us to identify **11** as a viable lead candidate for *in vivo* human studies.

Compound	A	B	C	Log P_{C18}	Ki*(nM) Aβ(1-40)	2 min uptake*(% ID-kg)/g	2/30 min ratio
1 [C-11]PIB	OH	H	$NH^{11}CH_3$	1.2	4.3[1]	0.21[1]	12[1]
2	OH	H	^{18}FPrNH	1.5	25	0.17	16
3	OH	H	$^{18}FPrNCH_3$	2.1	7.7	0.25	3.2
4	OH	H	$^{18}FEtNCH_3$	1.8	7.4	0.26	2.8
5	^{18}FPrNH	H	OH	1.6	28	0.24	13
6	^{18}FEtO	H	NH_2	1.7	7.2	0.30	2.5
7	^{18}FEtO	H	$NHCH_3$	2.4	3.2	0.22	2.3
8	$^{18}FEtO(EtO)_2$	H	$NHCH_3$	2.2	4.9	0.29	3.0
9	^{18}FEtO	H	OH	1.6	4.1	0.27	2.5
10	^{18}FEtO	H	OCH_3	2.9	1.2	0.29	2.6
11	OH	^{18}F	$NHCH_3$	1.7	5.9	0.31	8.4

* Average of triplicate determinations.



Conclusion: Preliminary results from *in vivo* human imaging studies using **11** have been encouraging as well, and further *in vivo* studies are planned to characterize **11** as a potential [F-18]-labeled tracer for Aβ plaque imaging studies.

Acknowledgement: NIA, DOE, GE Healthcare.

References: [1] Mathis et al., J. Med. Chem. 46:2740-2754 (2003). [2] Klunk et al., Ann. Neurol. 55:306-319 (2004). [3] Lopresti et al., J. Nucl. Med. 46:1959-1972 (2005).

Keywords: Benzothiazole, Amyloid Plaque, [F-18]PIB

IS AGING A REASON FOR THE FAILURE OF ^{11}C-PITTSBURGH COMPOUND B IN TRANSGENIC MICE MODEL?

D. BENDER[1], H. AUDRAIN[1], A.K. OLSEN[1], A. MØLLER[1], M. SKOVGAARD-JENSEN[2], M. WEST[2] and A. GJEDDE[1]

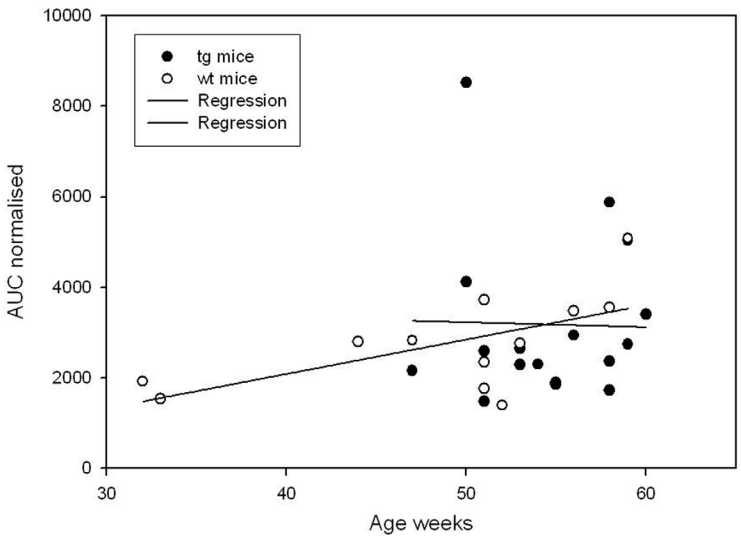
[1]PET Centre, Aarhus University Hospital, Aarhus, Denmark; [2]Institute for Anatomy, Aarhus University, Aarhus, Denmark

Introduction: ^{11}C-labelled Pittsburgh compound B (^{11}C-PIB) is used to image β-amyloid plaque in human by means of PET. Transgenic (tg) mice are used as animal model for the formation of β-amyloid plaque. So far, attempts to study β-amyloid plaque ^{11}C-PIB in tg models and μPET systems failed (W.E. Klunk et al., The Journal of Neuroscience, 2005; 25(46):10598 –10606; H. Toyama et al., Eur. J. Nucl. Med. Mol. Imaging 2005; 32 (5) 594–600), i.e. a statistical significant difference in ^{11}C-PIB retention between tg and wild type (wt) mice was not observed. A reason could be an age related decrease in cerebral blood flow. We were therefore interested in the effect of age on the retention of PIB.

Experimental: 19 PS1-A246E/APPswe tg mice and 13 wt controls (age 32 to 60 weeks) were PET scanned anaesthetized for 30 minutes in a Concorde μPET R4 PET scanner. When possible, tracer (15–35 MBq) was applied intravenously, otherwise intraperitoneal. Regions of Interest (ROI) were drawn manually according to a mice brain atlas and time-activity-curves (TAC) were generated using the Concorde Microsystems ASIPro software. Areas under the curves (AUC) divided by the mean activity at the end of scan were used to compare results.

Results and Discussion: TAC's for different ROI's showed no significant diferences. All results refer therefore to a ROI covering the whole brain. Whereas tg mice did not show an age dependency, wt mice showed an increase of AUC as a function of age, see figure.

Thus, reduced cerebellar blood flow in aging mice and by that an increased residence of tracer in the brain might contribute to the failure of PIB in μPET studies of tg mice. However, this effect might be only of minor importance. Dense-core plaques in the most common tg mice models are centered on vessel walls (S. Kumar-Singh et al., Am. J. of Pathology, 2005; 167 (2): 527–543). Thus, partial volume effects from excessive circulating residual radioactivity in the vessels might overshadow the signal of PIB bound to plaques. Consequently TAC's obtained in tg will not differ much from those obtained in wt mice which reflect only blood flow.



Conclusion: In conclusion a successful tracer for the μPET imaging of β-amyloid plaque in tg mice models should therefore show besides plaque binding a rapid clearance from blood.

Acknowledgement: This work was supported by Danish Agency for Science, Technology and Innovation grant 22-04-0514.

Keywords: Amyloid Plaque, PIB, Transgenic Mice, μPET