Table 3 Absorbed doses in the source organs

	Human			Mouse
	All subjects (n = 6)	Male (n = 3)	Female (n = 3)	
Organ				
Adrenal gland	1.96E01 ± 2.00	2.03E01	1.90E01	1.35E01
Brain	9.91 ± 1.82	8.95	1.09E01	4.17
Breasts	8.69 ± 2.55	6.68	1.07E01	9.90
Gallbladder wall	3.33E02 ± 2.51E02	2.16E02	4.50E02	1.68E01
Lower large intestine wall	2.52E01 ± 1.26E01	2.41E01	2.63E01	1.57E01
Small intestine	3.36E01 ± 3.07E01	2.07E01	4.64.E01	3.85E01
Stomach wall	1.61E01 ± 3.44	1.35E01	1.87E01	1.39E01
Upper large intestine wall	2.98E01 ± 1.50E01	2.36E01	3.59E01	1.83E01
Heart wall	1.62E01 ± 1.70	1.50E01	1.74E01	8.34
Kidneys	2.01E01 ± 4.30	1.85E01	2.17E01	1.32E01
Liver	7.75E01 ± 1.45E01	7.38E01	8.11E01	2.55E01
Lungs	1.46E01 ± 1.10	1.49E01	1.43E01	7.96
Muscle	1.03E01 ± 1.27	1.07E01	9.90	7.89
Ovary	1.67E01 ± 6.65	1.18E01	2.16E01	1.67E01
Pancreas	2.32E01 ± 3.11	2.17E01	2.47E01	1.45E01
Red marrow	1.31E01 ± 1.70	1.16E01	1.46E01	1.23E01
Osteogenic cells	1.60E01 ± 3.65	1.25E01	1.95E01	1.82E01
Skin	7.30 ± 1.39	5.99	8.60	8.70
Spleen	1.37E01 ± 2.48	1.27E01	1.48E01	7.83
Testis	7.32 ± 2.16	7.32	-	1.15E01
Thymus	1.00E01 ± 1.85	8.37	1.16E01	1.08E01
Thyroid	8.36 ± 1.38	8.86	7.86	1.10E01
Urinary bladder wall	2.23E01 ± 7.33	1.81E01	2.66E01	4.31E01
Uterus	1.67E01 ± 8.13	1.14E01	2.19E01	1.77E01
Total body	1.38E01 ± 1.63	1.22E01	1.53E01	1.22E01
Effective dose (μSv/MBq)	1.86E01 ± 3.74	1.64E01	2.09E01	1.48E01

Averaged absorbed dose estimates (μGy/MBq) for the target organs from the whole-body PET data (n = 6) from experiments involving human subjects of [^{18}F] FACT and mice of [^{18}F]FACT. Average absorbed dose for male subjects (n = 3).

The effective dose estimated from the human PET study was 18.6 ± 3.74 μSv/MBq. The effective doses of [^{18}F]FACT estimated from the clinical PET studies among other ^{18}F-labelled PET amyloid radioligands were as follows: [^{18}F]AV-45, 13 and 19.3 μSv/MBq [8,9]; [^{18}F] GE067, 33.8 μSv/MBq [10]; and [^{18}F]BAY94-9172, 14.67 μSv/MBq [11]. For PET analysis of [^{11}C]PIB, Scheinin et al. normalised the %ID using the ratio of individual and reference subjects' body weights (Equation 4) [7]. However, in the present study, we did not normalise the %ID data because there was a small difference between the effective dose with normalisation (17.6 ± 2.12 μSv/MBq) and the present effective dose (18.6 ± 3.74 μSv/MBq). Therefore, we concluded that body weight normalisation does not influence the effective dose.

The effective dose of [^{18}F]FACT from the mouse experiments (14.8 μSv/MBq) was underestimated as compared with that from the human subject PET studies (18.6 μSv/MBq) (Table 3). This discrepancy corresponded to 0.76 mSv (2.96 and 3.72 mSv from mice and humans, respectively) while assuming an injected activity of 200 MBq as a clinically relevant dose. The underestimation of absorbed dose in the mouse gallbladder (20 times lower) and liver (3 times lower) relative to the human PET studies may have been responsible for the underestimation of the effective dose. High absorbed doses in the liver, gallbladder and small intestine of mice indicated that the biodistribution pattern of [^{18}F]FACT in mice includes hepatobiliary excretion, as was observed in the PET scans involving human subjects. However,

the estimated absorbed dose in the gallbladder was 20 times lower than the estimate from human subject data sets because we could not remove the gallbladder of the mouse. Therefore, to evaluate the effective dose of [^{18}F] FACT in target organs, a whole-body PET scan of human subjects may be preferable as compared with the extrapolation from mouse experiments.

Clinical applicability of [^{18}F]FACT

The present whole-body PET study was performed using healthy elderly subjects and not patients with AD. Previously, Koole et al. speculated that if brain uptake of ^{18}F amyloid ligand increased by a factor of three, this will only influence estimation of the effective dose within 1%; however, when the subject had taken medication that changed the function of the hepatic metabolism, the estimated effective dose will vary with a larger range [10].

In the present series of PET studies, brain PET scans using the 3D mode were performed between the first and the second emission scan. Therefore, the injected dose for 2D whole-body scans was set to lower level than usual, and the averaged injected activity of 160.8 MBq corresponded to a radiation dose of 2.99 mSv per single administration. With regard to the optimal injected activity that can ensure sufficient image quality for clinical use, the peak noise-equivalent counts ratio (NECR) is often used in its determination. It has also been reported that the peak NECR in 2D mode was not reached with an acceptable range of injected activity, whereas in 3D mode, there was a distinct maximum for the NECR for which the corresponding injected activity was based on patient height and weight [19]. For the scanner used in our study, the NECR peak in 3D mode was reached at 4.44 kBq/ml using an 8,000-ml phantom [13]. When the subject's height and weight were assumed to be 170 cm and 60 kg, respectively, this assumption corresponded to the optimal injected dose of about 260 MBq. In a real situation, there exists the effect of the activity outside the axial FOV, and the optimal injected dose would be much lower. Injected activity indicates radiation dose; for example, 200 MBq indicates a radiation dose of 3.72 mSv. ICRP 62 [20] recommended that the maximum radiation dose that causes a 'minor to intermediate' increase of risk levels while preserving social benefit levels that are 'intermediate to moderate' has an effective limit of 10 mSv/year [20,21]. Thus, the maximum injectable activity is 537.6 MBq [^{18}F]FACT/year, and this injection dose limit allows two or three PET scans to be performed. Furthermore, amyloid imaging is mainly undertaken in elderly patients aged >50 years, even though for early detection of AD, patients aged <50 years will also have an amyloid PET scan. According to the guidance on medical exposures in medical and biomedical research by the European Commission [22], dose restrictions for patients aged over >50 years are not as strict as for younger patients. Therefore, considerably more multiple PET scans may be possible.

Conclusions

The effective dose of the ^{18}F-labelled amyloid imaging agent, [^{18}F]FACT, was found to be acceptable for clinical study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MS carried out the data analysis and interpretation and drafted the manuscript. TM, HT, YK and KY performed the study design and contributed to the intellectual discussion. NO, SF and RI performed the animal experiments and synthesis of PET probes. KF, SW, KH, MM and HA provided the clinical data. All authors read and approved the final manuscript.

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Novel ^{18}F-labeled arylquinoline derivatives for non-invasive imaging of tau pathology in Alzheimer's disease

Short title: Novel ^{18}F-labeled tracer for imaging tau

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Abstract

Neurofibrillary tangles in Alzheimer's disease (AD) brains are composed of the microtubule-associated protein tau. Non-invasive monitoring of tau protein aggregates in the living brain will provide useful information regarding tau pathophysiology in AD. However, no positron emission tomography (PET) probes are currently available for selective detection of tau pathology in AD. We have previously reported ^{18}F-labeled THK-523 as a candidate of PET tau imaging radiotracer. Following compound optimization, we developed novel ^{18}F-labeled arylquinoline derivatives, ^{18}F-THK-5105 and ^{18}F-THK-5117, for use as tau imaging PET tracers.

Methods: ^{18}F-labeled compounds were prepared from the corresponding tosylated precursors. *Binding affinity of compounds to synthetic tau aggregates and tau-rich AD brain homogenates* was determined by saturation and competition binding assays. Binding selectivity of compounds to tau pathology was evaluated by autoradiography of AD brain sections. Pharmacokinetics of THK compounds was assessed in biodistribution studies in normal mice. A 14-day toxicity study with intravenous administration of THK compounds was performed using rats and mice.

Results: *In vitro* binding assays demonstrated higher binding affinity of THK-5105 and THK-5117 to tau protein aggregates and tau-rich AD brain homogenates, compared to THK-523. Autoradiographic analyses of AD brain sections showed that these radiotracers preferentially bound to neurofibrillary tangles and neuropil threads, which co-localized with

Gallyas-positive and immunoreactive tau protein deposits. The distribution of these radiotracer binding in AD brain sections was completely different from that of [^{11}C]PiB, showing preferential binding to amyloid plaques. Furthermore, these derivatives demonstrated abundant initial brain uptake and faster clearance in normal mice than ^{18}F-THK-523 and other reported ^{18}F-labeled radiotracers. THK-5105 and THK-5117 showed no toxic effects related to the administration of these compounds in mice and rats and no significant binding for various neuroreceptors, ion channels and transporters at 1 μM concentrations. **Conclusion:** ^{18}F-labeled THK-5105 and THK-5117 are promising candidates as PET tau imaging radiotracers.

Key Words

Alzheimer's disease, tau, neurofibrillary tangles, positron emission tomography, molecular imaging

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. At present, approximately 18 million people worldwide suffer from AD, and this number is estimated to double by 2025(*1*). The major pathological hallmarks of AD are senile plaques (SPs) and neurofibrillary tangles (NFTs). SPs are composed of amyloid-β protein (Aβ), a 39–43 amino acid protein product derived from the proteolytic cleavage of the amyloid precursor protein (APP). Abnormalities in the production or clearance of Aβ are considered to be the initiating events in AD pathogenesis(*2*). Excessive Aβ concentrations lead to its aggregation and formation of SPs, followed by NFT formations, synaptic dysfunction, and neuronal death. NFTs are composed of hyperphosphorylated tau, a microtubule-associated protein that stabilizes microtubule assembly in axons(*3*). Tau accumulation is also recognized as neuropil threads and dystrophic neurites in the AD brain(*4*). Phosphorylation of tau decreases its ability to bind to microtubules, which are destabilized, leading to neuronal death. NFT lesions follow a stereotypical pattern, initially appearing in the transentorhinal cortex, followed by the entorhinal cortex and hippocampus, and subsequently the neocortex(*5*). In AD patients, the severity of tau pathology is closely related to neuronal loss(*6, 7*) and cognitive impairment(*8, 9*). The deposition of NFTs is thought to begin before extensive neuronal loss and cognitive decline occur. Thus, non-invasive detection of tau pathology would be useful to predict future cognitive

decline in the preclinical stages of AD as well as to track disease progression before extensive neuronal loss occurs.

Several researchers have focused on developing radiotracers for imaging tau pathology in the human brain(*10-17*). Tau imaging radiotracers need to cross the blood-brain barrier and have a high binding affinity to NFTs with minimal nonspecific binding(*18*).

2-(1-(6-[(2-^{18}F-Fluoroethyl)(methyl)amino]-2-naphthyl)ethylidene)malononitrile (^{18}F-FDDNP) is claimed as the only PET tracer that allows measurement of the amount of tau protein deposits in human brain(*19*). However, ^{18}F-FDDNP was found to have lower binding affinity for protein fibrils relative to [^{11}C]Pittsburg compound B (PiB)(*20*)·(*21*). In addition, this tracer has been claimed to bind to both SPs and NFTs equally(*22*). In the neocortex of AD brain, SPs and NFTs co-localize with each other, where Aβ concentrations are 5–20 times higher than that of tau(*23, 24*). In such cases, the signal from the SPs would be so overwhelming that it would obscure the signal from the NFTs. Therefore, the development of selective tau imaging tracers is necessary for accurate and quantitative evaluation of tau pathology in AD.

In the past few years, we also have screened over 2000 compounds to develop novel radiotracers with high affinity and selectivity for tau aggregates. Consequently, we identified a series of novel quinoline and benzimidazole derivatives that bind NFTs, and to a lesser extent Aβ plaques(*10*). Serial analyses of these compounds led to the design and synthesis of the novel

tau imaging agent, ^{18}F--6-(2-fluoroethoxy)-2-(4-aminophenyl)quinoline (^{18}F-THK-523)(*15, 17*). Preclinical analyses of ^{18}F-THK-523 indicated that this tracer selectively labels tau pathology in AD brain. However, the preclinical data suggest that the pharmacokinetics and binding characteristics of ^{18}F-THK-523 might not reach the required optimal levels required for PET tracers. Through our optimization process, we developed novel ^{18}F-labeled 2-arylquinoline derivatives that are promising candidates for *in vivo* tau imaging probes. In this study, we performed the preclinical evaluation of the binding and pharmacokinetic properties of these compounds.

MATERIALS AND METHODS

Synthesis and Radiosynthesis of 2-Arylquinoline Derivatives

The chemical structures of

6-[(3-^{18}F-fluoro-2-hydroxy)propoxy]-2-(4-dimethylaminophenyl)quinoline (^{18}F-THK-5105)

6-[(3-^{18}F-fluoro-2-hydroxy)propoxy]-2-(4-methylaminophenyl)quinoline (^{18}F-THK-5117),

6-[(3-^{18}F-fluoro-2-hydroxy)propoxy]-2-(4-aminophenyl)quinoline (^{18}F-THK-5116) and

^{18}F-THK-523 are shown in Figure 1. ^{18}F-THK-5105, ^{18}F-THK-5116, and ^{18}F-THK-5117 were prepared from the corresponding tosylate precursors according to the scheme as indicated in the Figure 2. Details on their syntheses will be described elsewhere (S. Furumoto et al, in

preparation). Briefly, the aqueous $^{18}F^-$ contained in K_2CO_3 solution (1.59–3.69 GBq) and Kryptofix222 (15 mg) were placed in a brown vial. Water was removed by azeotropic evaporation with acetonitrile. After drying, the activated ^{18}F-KF/Kryptofix222 was reacted with the precursor (3 mg) in DMSO (0.7 mL) at 110°C for 10 min. Then, 2 M HCl was added to the solution followed by additional 3 min-reaction for deprotection of the hydroxyl group. After neutralization with 4 M AcOK, the product was purified by semi-preparative high-performance liquid chromatography (HPLC; column: Inertsil® ODS-4 (GL Sciences, Inc., Tokyo, Japan); mobile phase: 20 mM NaH_2PO_4/acetonitrile (55/45 for THK-5105 and -5117, 65/35 for THK-5116); flow rate: 5.0 mL/min). The radiolabeled product was dissolved in ethanol, DMSO, or saline with polysobate-80 (<0.1%) for biological evaluation.

^{18}F-THK-523 and ^{18}F-FDDNP were also prepared in a similar manner described above using the corresponding tosylate precursors reported previously(*15, 25, 26*). Radiolabeling of [^{11}C]PiB was performed using its precursor (2-(4-aminophenyl)-6-methoxymethoxybenzothiazole) and [^{11}C]methyl triflate, as previously described(*27*).

Determination of Log P Values

Determination of Log P values was carried out by HPLC method according to the OECD guideline with slight modification. Briefly, 12 reference compounds whose Log P values range

between 0.5 and 4.0 were analyzed by HPLC under the following conditions; HPLC: a JASCO LC-2000 Plus series (JASCO, Tokyo, Japan); column: Inertsil® ODS-4 (4.6 × 150 mm, 5 μm; GL Sciences, Inc., Tokyo, Japan); mobile phase: 20 mM NaH_2PO_4 (pH 7.4)/acetonitrile (55/45); flow rate: 1.5 mL/min; UV absorbance: 245 nm; column temperature: 40°C. Then, a calibration curve of Log (tR-t0) (tR: retention time; t0: dead time) versus log P of each reference compound was created (R^2 = 0.9469). Test compounds listed in Table 2 were also analyzed by the same HPLC method to measure Log (tR-t0) values that were used for determination of Log P values from the calibration curve.

In Vitro Binding Assays

Synthetic human Aβ1-42 was purchased from Peptide Institute Inc. (Mino, Japan). Recombinant K18ΔK280-tau protein was obtained from Life Technologies Japan Ltd. (Tokyo, Japan). Fibrils of Aβ1-42 and K18ΔK280-tau were prepared as described previously(*15*). Briefly, synthetic Aβ1-42 (200 μM) and K18Δ280K-tau (20 μM) solutions in phosphate buffered saline (PBS) were incubated at 37°C with agitation for 3-4 days. We additionally prepared AD brain homogenates for binding assay, because the structural conformation of synthetic protein fibrils does not fully correlate with the structure of native protein deposits in the human brain. Human brain tissue was isolated from a mesial temporal frozen sample of an AD patient and

homogenized in PBS. Brain tissue homogenates were aliquoted and frozen at -80°C until used. Insoluble Aβ and tau levels were determined using a human β-amyloid enzyme-linked immunosorbent assay (ELISA) kit (Wako, Osaka, Japan) and a human tau ELISA kit (Life Technologies Japan Ltd., Tokyo, Japan), respectively. Next, brain homogenates and the solutions of synthetic Aβ1-42 or K18Δ280K-tau fibrils were incubated with increasing concentrations of ^{18}F-THK-5105 (0.1–250 nM). To account for non-specific binding of ^{18}F-THK-5105, the reactions were performed in triplicate in the presence of 2 μM unlabeled THK-5105. The binding reactions were incubated for 1 hour at room temperature in assay buffer (Dulbecco's PBS; 0.1% bovine serum albumin [BSA]). Bound radioactive compounds were separated from free radioactive compounds by filtration under reduced pressure (MultiScreen HTS Vacuum Manifold; Millipore, Billerica, MA, USA). Filters were washed thrice with assay buffer, and the radioactivity contained within the filters was counted in a γ-counter (AccuFLEX γ7000, Aloka, Tokyo, Japan). Binding data were analyzed using a curve fitting software that calculates the K_D and B_{max} using non-linear regression (GraphPad Prism, GraphPad Software, San Diego, CA).

For inhibition studies, the assay buffer containing each compound (0.1–1000 nM), ^{18}F-THK-5105 (1.76 nM, approx. 37 kBq), K18Δ280K-tau (200 nM), and 0.1% BSA was incubated at room temperature for 1 h. Non-specific binding was determined in the presence of

10 μM THK-5105. The mixture was filtered through Multiscreen HTS 96-well filtration plates followed by washing thrice with PBS (0.1% BSA), and the filters containing bound ^{18}F ligand were counted in a γ-counter. Percentage of bound radioligand at each concentration was measured in triplicate and then plotted against the inhibitor concentration. Half-maximal inhibitory concentration (IC50) values were determined from the displacement curves using the GraphPad Prism software. Inhibition constant (Ki) values were calculated from the IC50 values using the Cheng-Prusoff equation(*28*).

Tissue Staining

Experiments were performed under the regulations of the ethics committee of Tohoku University School of Medicine. Paraffin-embedded hippocampal brain sections from an autopsy-confirmed AD case (78-year-old woman) were used for tissue staining with THK-5105. Brain sections were obtained from Fukushimura Hospital (Toyohashi, Japan). After deparaffinization, autofluorescence quenching was performed as previously described(*29*). Quenched tissue sections were immersed for 10 min in 100 μM THK-5105 solution containing 50% ethanol. Sections were then dipped briefly into water, rinsed in PBS, coverslipped with FluorSave Reagent (Calbiochem, Darmstadt, Germany), and examined using a Nikon Eclipse microscope (Tokyo, Japan) equipped with a blue-violet filter (excitation 400–440 nm, dichroic

mirror 455 nm, barrier filter 470 nm). Sections stained with THK-5105 were subsequently immunostained with the AT8 anti-Tau antibody (Innogenetics, Ghent, Belgium; diluted 1:20). Following incubation at 4°C in the primary antibody for 16 h, sections were processed by the avidin-biotin method using a Pathostain ABC-POD(M) Kit (Wako) and diaminobenzidine as a chromogen. Sections were additonally stained using a modified Gallyas-Braak method (pretreatment with 0.3% potassium permanganate for 10 min, followed by 0.1% oxalic acid for 3 min)(*30*).

Autoradiography of Human Brain Sections

For the autoradiographic study, 8 μm-thick paraffin-embedded brain sections from a healthy control (62-year-old man) and two AD patients (69-year-old man and 92-year-old woman) were used. After deparaffinization, sections were incubated for 10 minutes at room temperature with 0.5 MBq/ml radiolabeled compounds and washed briefly with water and 50% ethanol. After drying, the labeled sections were exposed overnight to a BAS-III imaging plate (Fuji Film, Tokyo, Japan). The autoradiographic images were obtained using a BAS-5000 phosphoimaging instrument (Fuji Film). The neighboring sections were stained using a modified Gallyas-Braak method or immunostained using the AT8 anti-tau monoclonal antibody (diluted 1:20; Innogenetics), the 4G8 Aβ antibody (diluted 1:100; Signet, Dedham, MA) or the 6F/3D Aβ

antibody (diluted 1:50; Dako, Glostrup, Denmark). For correlational analysis of the autoradiographic and immunohistochemical images, 36 circular regions of interest (ROIs) (the area of each ROI was approximately 7 mm^2) were placed on the gray matter of hippocampus, parahippocampal gyrus, fusiform gyrus, temporal gyri (superior, middle and inferior), insula, pre- and post-central gyri, superior frontal gyrus, paracentral lobule and cingulate gyrus. The percent area of positive signals in each ROI was calculated using ImageJ software. A correlational analysis between percent areas of tracer binding and positive immunostaining was performed using Pearson's simple correlation.

Biodistribution in Mice

The experimental protocol of animal study was approved by the Ethics Committee of Tohoku University School of Medicine. ^{18}F-labeled tracers (1.1–6.3 MBq) were injected into the tail vein of male ICR mice (n = 20, mean weight, 28–32 g). Mice were then sacrificed by decapitation at 2, 10, 30, 60, and 120 min post injection (p.i.). The brain, blood, liver, kidney, and femur were removed and weighed, and radioactivity was counted with an automatic γ-counter. The percent injected dose per gram of tissue (%ID/g) was calculated by comparing tissue counts to tissue weight. Each %ID/g value is expressed as a mean ± SD of 4 separate experiments.

Animal Toxicity Studies

A 14-day toxicity study with intravenous administration of a single dose of THK-5105 and THK-5117 was performed using Sprague Dawley rats and ICR mice. Briefly, the study included 3 groups of male and female rats and mice that were administered 0 (Group 1), 0.1 (Group 2), and 1 (Group 3) mg/kg of test article (10% DMSO/90% distilled water) per rat or mouse by intravenous injection on study day 1. The study included clinical observations plus body weight measurements for a 14-day observation period. Hematology and pathological examinations were conducted on study day 2 and day 15. Detailed necropsies with external examinations were also performed.

Receptor Binding Assays

Receptor binding screens were conducted by Sekisui Medical Inc. (Tokyo, Japan). Binding inhibition effects of 1 μM THK-5105 and THK-5117 were evaluated in competitive radioligand assays against 60 common neurotransmitter receptors, ion channels and transporters. Percentage inhibition ratios were calculated by the following equation: inhibition ratio (%) = [1 – (B - N) / (B_0 - N)] × 100, where N is the non-specific bound radioactivity, and B and B_0 are the bound

radioactivity in the presence and absence of tested compounds, respectively. Data are expressed as the mean values of duplicate samples.

RESULTS

Radiosynthesis

All radiolabeled compounds were obtained in >97% radiochemical purities after HPLC purification. The decay corrected average radiochemical yields of ^{18}F-THK-523, ^{18}F-THK-5105, ^{18}F-THK-5116, ^{18}F-THK-5117, and ^{18}F-FDDNP were 58%, 48%, 41%, 48%, and 22%, respectively. The specific activities of ^{18}F-labeded compounds ranged from 37 to 110 GBq/μmol, corrected at the end of synthesis. The mean specific activity of [^{11}C]PiB was 35 GBq/μmol.

In Vitro Binding Assays

The binding properties of phenylquinoline derivatives to tau fibrils was investigated and compared to Aβ1-42 fibrils. Although only a single class of ^{18}F-THK-5105 binding sites was identified on Aβ1-42 fibrils, two classes of ^{18}F-THK-5105 binding sites were identified on K18Δ280-tau fibrils. As shown in Table 1, the K_d for the first class of K18Δ280-tau binding sites was 1.45 nM, indicating higher binding affinity to tau fibrils than to Aβ1-42 fibrils (K_d =

35.9 nM). Further, competitive binding assays with ^{18}F-THK-5105 displayed high binding affinity of phenylquinoline derivatives to tau fibrils (Figure 3). The K_i for THK-5117 was 10.5 nM, indicating that compared to THK-523 (K_i = 59.3 nM), THK-5117 has higher binding affinity for tau fibrils. In contrast, the K_i for FDDNP was 263 nM. In binding assays using mesial temporal brain homogenates containing high density of tau (1075 pmol/g) and moderate density of Aβ (434 pmol/g), both ^{18}F-THK-5105 (K_d = 2.63 nM, B_{max} = 358 pmol/g tissue) and ^{18}F-THK-5117 (K_d = 5.19 nM, B_{max} = 338 pmol/g tissue) showed higher affinity for mesial temporal brain homogenates, compared to ^{18}F-THK-523 (K_d = 86.5 nM, B_{max} = 647.1 pmol/g tissue) (Figure S1).

Tissue Staining and Autoradiography

The selective binding ability of the compounds was further examined using AD brain sections. The fluorescent compound THK-5105 clearly stained NFTs and neuropil threads in the hippocampal section of an AD patient (Fig. 4A). Selective binding of this compound with tau pathology was confirmed by comparing with the results of tau immunohistochemistry for the same sections (Fig. 4B). In contrast, SPs were faintly stained with THK-5105. Further, we compared findings of THK-5105 staining with those of Gallyas-Braak silver staining, a conventional technique used to visualize tau pathology in AD brain (Fig. 4C,D), and the binding

of THK-5105 to NFTs and neuropil threads was confirmed. The images of staining with THK-5116 and THK-5117 were similar to those with THK-5105 (data not shown).

To investigate the binding ability of ^{18}F-THK-5105 and ^{18}F-THK-5117 to NFTs at tracer doses, *in vitro* autoradiography was performed in postmortem AD brain sections, and the findings were compared with Gallyas-Braak staining and immunohistochemistry. In the mesial temporal sections, laminar distributions of ^{18}F-THK-5105 and ^{18}F-THK-5117 were observed in the deep layer of gray matter (Fig. 5A). A high density of tracer accumulation was observed in the CA1 area of the hippocampus (arrowheads in Fig. 5), which is reported as the most frequent site for NFTs in AD(*31*). These tracer distributions coincided with Gallyas-Braak staining and tau immunostaining (Fig. 5B), but not with the distribution of [^{11}C]PiB (Fig. 5A) and Aβ immunostaining (Fig. 5B). In contrast, no significant accumulation of ^{18}F-THK-5105 and ^{18}F-THK-5117 was observed in the hippocampus of the healthy control subject (Fig. S2). ^{18}F-THK-5116 failed to give a specific signal in the AD brain sections (data not shown).

To further assess the regional differences of tracer binding in AD brain, ^{18}F-THK-5105 autoradiography was conducted using AD hemibrain sections and compared with the Aβ PET tracers PiB(*32*). ^{18}F-THK-5105 densely accumulated in the gray matter of the hippocampus, parahippocampal gyrus, fusiform gyrus, inferior and middle temporal gyri, insula, and cingulate gyrus (Fig. 6A), regions known for the abundance of tau pathology in AD(*33*). In contrast,