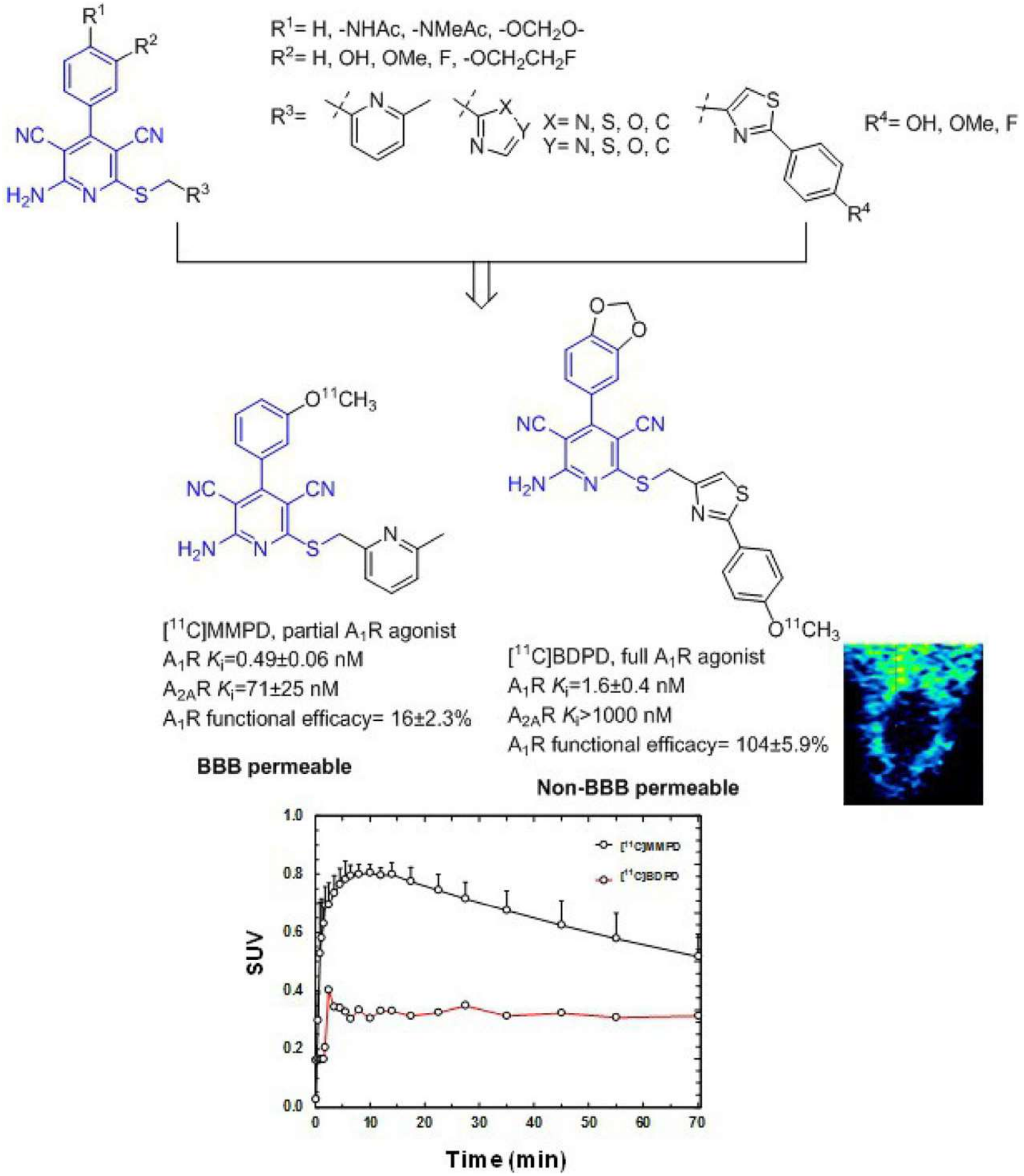

Poster Category: Radiolabeled Compounds - Neuro Sciences

P-244 | Fluorine-18 radiolabelling and in vitro/in vivo metabolism of [^{18}F]D4-PBR111

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Objectives

The 18 kDa Translocator Protein (TSPO) is a receptor protein located in the outer mitochondrial membrane.[1,2]

TSPO is a bio-marker for inflammation associated with numerous diseases including cancer, multiple sclerosis, Parkinson's and Alzheimer's diseases, stroke, Huntington's disease, and HIV encephalitis.[3,4] Consequently, there is significant interest in radiolabelled TSPO ligands as new radiotracers. This includes [^{18}F]PBR111 which shows potential for imaging neuroinflammation[5,6] but suffers from significant de-fluorination in vivo (rats). This leads to non-specific bone uptake and low signal-to-noise ratios in vivo, leading to lower quality PET images. To address these problems, a deuterated 2nd generation radiotracer has been synthesised and its metabolic stability compared to regular [^{18}F]PBR111.

Methods

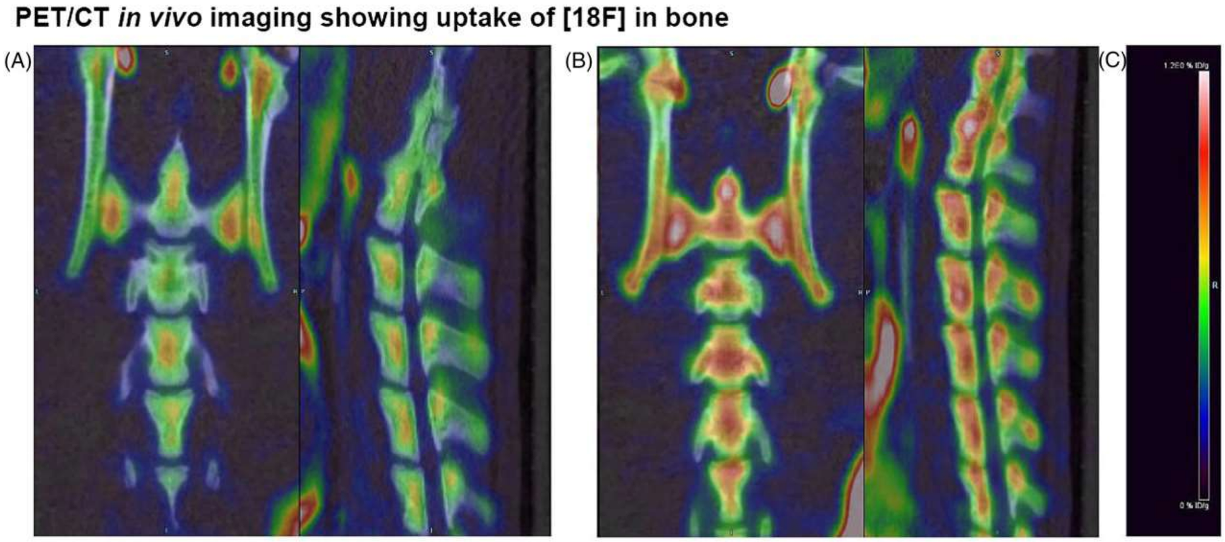
The synthesis of [^{18}F]D4-PBR111 radiolabelling precursor was achieved following an adaptation of our previously published method.[5] The radio-synthesis of [^{18}F]PBR111 and [^{18}F]D4-PBR111 was performed on a Synthra synthesis module by nucleophilic substitution of PBR111 or D4-PBR111 tosylate precursor with [^{18}F]fluoride based on adaptations of conditions previously described.[5] In vitro metabolism was evaluated by incubating [^{18}F]PBR111 or [^{18}F]D4-PBR111 with either rat or human liver microsomes, NADPH generating solution and 0.1M potassium phosphate buffer pH 7.4 (PBS) at 37°C. Supernatant was collected at various time points over a 60 min period. The supernatant was then analysed via radio-HPLC to determine the metabolite components. A control sample was used for each assay containing all the components of the assay apart from the NADPH generating solution to rule out any breakdown of the radiotracer not caused by the microsomal process. In vivo PET and metabolite studies consisted of male Sprague Dawley Rats (n = 16) being injected with either 100 MBq [^{18}F]PBR111 or [^{18}F]D4-PBR111 and a PET acquisition was performed for a 60 min scan. During this process blood samples were taken from the rats at various time points up to 60 min via the femoral artery. Plasma was separated from whole blood and the percentage of the metabolite components and the in-tact tracer was analysed by radio-HPLC and Solid Phase Extraction (SPE) as previously described.[5]

Results

In vitro assay results showed the presence of 7 visible [^{18}F] radio-metabolite peaks and were given a numerical value based on their polarity on the HPLC chromatogram. The results showed that rat microsomes metabolised both [^{18}F]PBR111 and [^{18}F]D4-PBR111 much faster than the human microsomes. In vivo PET imaging in rats showed a 42% reduction of the median [^{18}F]D4-PBR111 (Figure 1, A) uptake in bone (vertebrae) compared to non-deuterated [^{18}F] PBR111 (Figure 1B). This data supports the hypothesis that the introduction of deuterium has significantly reduced the de-fluorination of the PBR111 radiotracer.

Conclusions

A deuterated radiotracer [^{18}F]D4-PBR111 was developed and evaluated in vivo in rats, demonstrating that it is more resistant to metabolic breakdown compared to non-deuterated [^{18}F]PBR111. Careful choice of the site of deuteration resulted in a decreased rate of de-fluorination, and a notable increase in the median uptake of the radiotracer in regions with high TSPO expression. Rat and human liver microsomal assays were an effective screening tool for predicting the potential metabolic differences between the deuterated and non-deuterated analogues. Our results provide further evidence of the benefit that deuterium can have, not only, in stabilisation but also in altering the metabolic profile of a radiotracer. Further studies are now underway to evaluate [^{18}F]D4-PBR111 vs [^{18}F]PBR111 in animal disease models.


PET/CT *in vivo* imaging showing uptake of [18F] in bone

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Poster Category: Radiolabeled Compounds - Neuro Sciences

P-245 | A novel imaging probe with selectivity for tau-oligomeric protein aggregates: In vitro evaluation and radiolabelling with fluorine-18

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Objectives

Soluble, oligomeric aggregates of misfolded proteins like amyloid beta (Aβ) and tau are neurotoxic and cause damage to neurons in the early stages of neurodegenerative diseases before the development of cognitive symptoms.[1] At present, we lack selective molecular tools to image and interrogate the formation of soluble oligomers of Aβ and tau in vivo. Such molecular tools, and their translation into PET ligands would facilitate our understanding of the origin and progression of neurodegenerative diseases. Towards this goal, we have developed pTP-TFE, a fluorescent oligothiohene compound containing several fluorine atoms, which would enable imaging applications using ^{19}F MRI and ^{18}F PET. Herein, we describe the in vitro behaviour of pTP-TFE and progress towards radiolabelling pTP-TFE with fluorine-18 for in vivo PET studies.

Methods

To investigate the ability of pTP-TFE to detect small, soluble aggregates, pTP-TFE, its analogue pFTAA[2] and thioflavin-T were each incubated with Aβ monomer and tau monomer at 37°C at pH 7.4. The change in fluorescence was measured over the course the aggregation reaction, and the species present at each timepoint were confirmed by transmission electron microscopy. (TEM) The affinity (K_d) of pTP-TFE the different sized aggregates of Aβ and tau were also measured. For radiolabelling with fluorine-18, [^{18}F]fluoride was azeotropically dried to afford [^{18}F]KF•K$_{222}$•K$_2$CO$_3$ complex using standard methods. Addition of [^{18}F]KF•K$_{222}$•K$_2$CO$_3$ to difluoroiodomethane in MeCN gave [^{18}F]fluoroform, that was distilled from the reaction mixture. [^{18}F]Fluoroform was trapped in DMF at −60°C, before KO^{t}Bu and 2-bromothiophenecarboxaldehyde were added at room temperature. HPLC of the reaction mixture spiked with authentic standard was used to confirm formation of the desired ^{18}F-labelled product.

Results

ThT fluorescence increases at the late stages of the aggregation of Aβ and tau, as β-sheet fibrillar aggregates of the proteins form. In contrast, the fluorescence of pFTAA increases early in the aggregation reaction, when TEM confirms the presence of oligomers of the proteins at these timepoints. pTP-TFE behaves similarly to pFTAA; however, the increase in fluorescence occurs earlier during the aggregation reactions of Aβ and tau, suggesting that pTP-TFE binds even smaller oligomeric aggregates than pFTAA. Measurements of the binding affinity (K_d) of pTP-TFE to aggregates of Aβ and tau of increasing size showed that pTP-TFE had a K_d = 60 nM for the tau-oligomer rich fraction, while smaller and larger tau aggregates and all fractions of Aβ have a $K_d \approx 1$ μM. This suggests that pTP-TFE is a probe selective for binding to tau oligomers and is a promising candidate for radiolabelling with fluorine-18 for PET imaging of oligomeric tau in vivo. Towards this end, [^{18}F]-1-(5-bromothiophen-2-yl)-2,2,2-trifluoroethan-1-ol was identified as the key intermediate towards the synthesis of [^{18}F]pTP-TFE. Vugts et al,[3] described the synthesis of aryl [^{18}F] trifluoroethanols by reaction of an aldehyde with [^{18}F] fluoroform-derived [^{18}F]CF$_3^-$. Using this method, [^{18}F] fluoroform was isolated in 25 % (n = 6) n.d.c. radiochemical yield (RCY), and [^{18}F]-1-(5-bromothiophen-2-yl)-2,2,2-trifluoroethan-1-ol was synthesised in 66% crude RCY from [^{18}F]fluoroform and the corresponding aldehyde. Conditions for the Suzuki coupling of this fragment with the appropriate boronic ester precursor to give [^{18}F] pTP-TFE are ongoing.

Conclusions

We have developed a novel pTP-TFE, an oligothiophene derivative that binds to tau oligomers with high affinity and selectivity. To our knowledge, we believe this to be the first probe with this selectivity. Radiolabelling p-TP-TFE with fluorine-18 is ongoing, and [^{18}F]-1-(5-