Fluorine-18 Radiolabelling and in vitro / in vivo Metabolism of [^{18}F]D4-PBR111

Naomi A. Wyatt[1], Mitra Safavi-Naeini[1, 3], Andrew Wotherspoon[3], Andrew Arthur[3], An P. Nguyen[3], Arvind Parmar[3], Hasar Hamze[3], Charmaine M. Day[2], David Zahra[3], Lidia Matesic[2], Emma K. Davis[3], Gita L. Rahardjo[3], Nageshwar R. Yepuri[4], Rachael K. Shepherd[1], Rhys B. Murphy[4], Tien Q. Pham[2], Vu H. Nguyen[1, 3], Paul D. Callaghan[1, 3], Peter J. Holden[4], Marie-Claude Gregoire[1], Tamim A. Darwish[4], **Benjamin H. Fraser*** [1, 2]

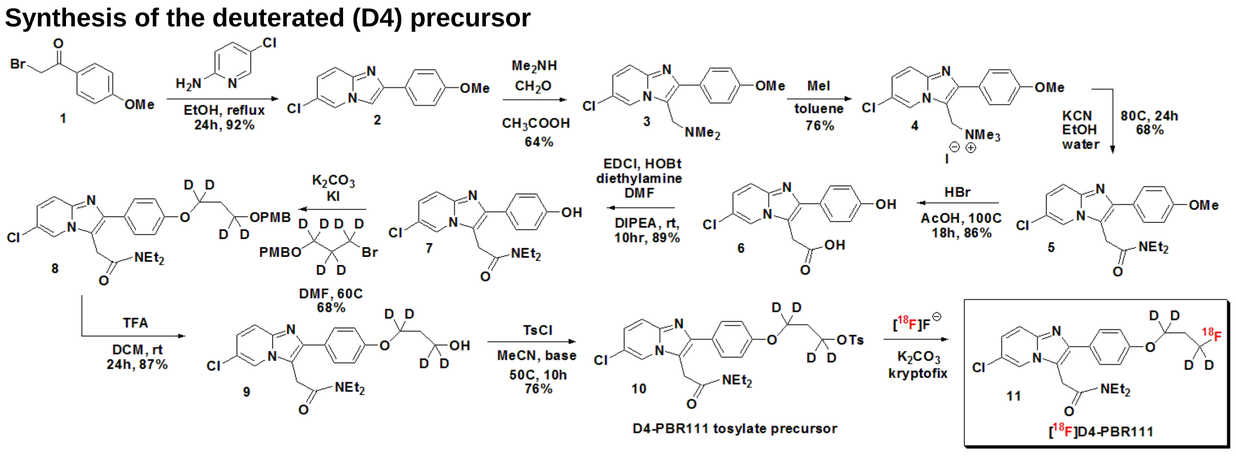
[1]Human Health Research, [2]Radioisotopes and Radiotracers, [3]Radiobiology and Bio-imaging, [4]National Deuteration Facility, The Australian Nuclear Science and Technology Organisation, Sydney, Australia ***benjamin.fraser@ansto.gov.au***

Introduction

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

Synthesis of the deuterated (D4) precursor



The synthesis of [^{18}F]D4-PBR111 radiolabelling precursor was achieved following an adaptation of our previously published method.[5] The key deuterated synthon was introduced by alkylation of the phenol **7** to give ether **8** in good yield (68%). Hydrolysis of the acetate group of **8** by treatment with cesium carbonate in methanol gave alcohol **9** (87%) which was then reacted with tosyl chloride to provide the tosylate radiolabelling precursor **10** in good yield (76%).

Radiopharmaceutical Preparation

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 conditions previously described.[5]

In vitro microsomal assays

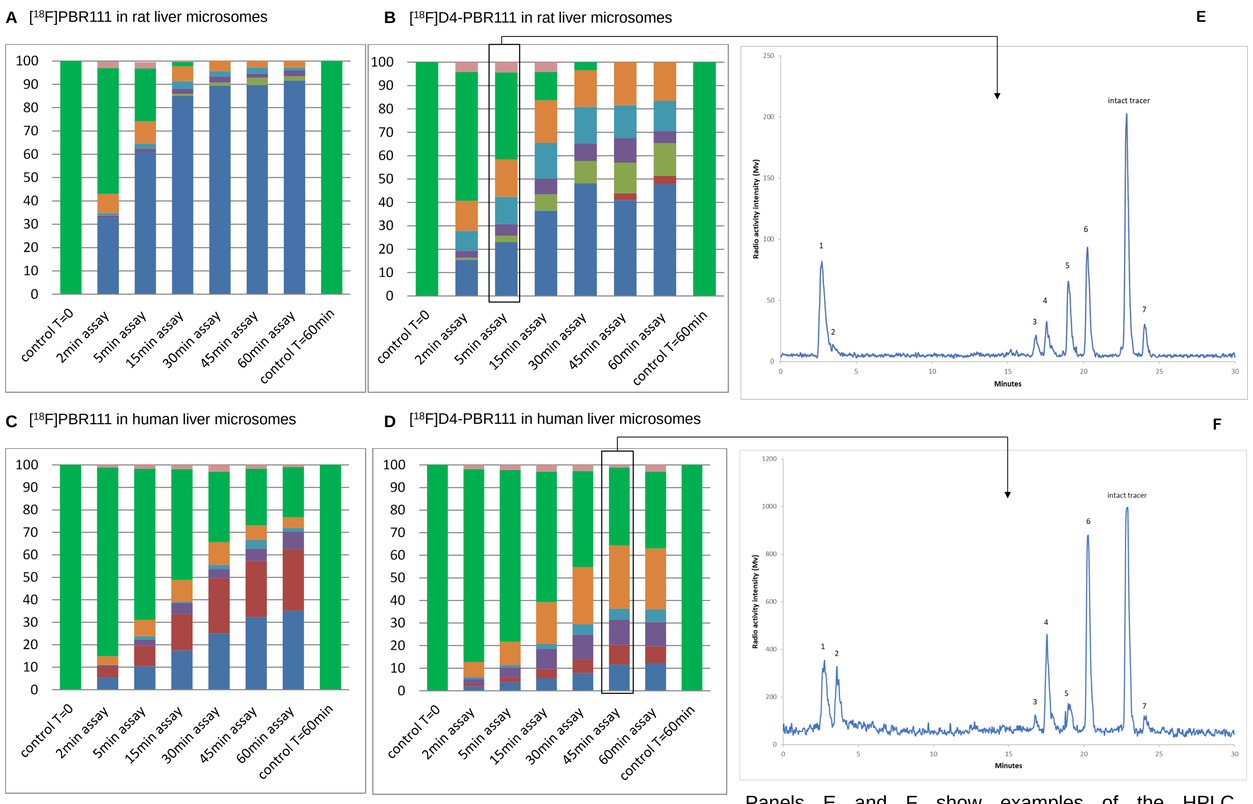
100μM of [^{18}F]PBR111 or [^{18}F]D4-PBR111 was incubated with either rat or human liver microsomes, NADPH generating solution (of 5mM G6P, 1mM NADP+, G6P-dehydrogenase 2 UI/assay), and 0.1M potassium phosphate buffer pH 7.4 (PBS) at 37^{o}C. Supernatant was collected at various time points over a 60min 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 imaging and metabolite study

Male Sprague Dawley Rats (n=16) were injected with either 100MBq [^{18}F]PBR111 or [^{18}F]D4-PBR111 and a PET acquisition was performed for a 60 minutes scan. During this process blood samples were taken from the rats at various time points up to 60mins 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] All *in vivo* experiments were approved by the ANSTO Animal Care and Ethics Committee prior to commencement.

Results

In vitro metabolism of [^{18}F]PBR111 vs [^{18}F]D4-PBR111 in rat and human microsomes



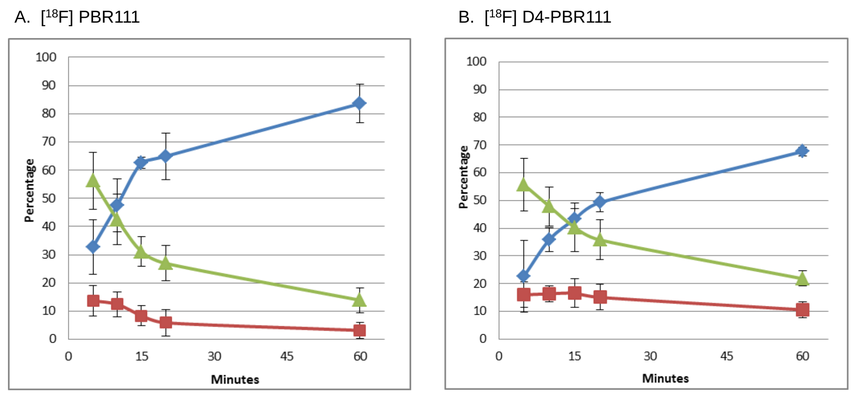
Radio-HPLC analysis showing the percentage of radioactive metabolites and in-tact tracer following incubation with rat and human microsomes over a 60min time course.

Panels E and F show examples of the HPLC chromatograms at particular time points in the microsomal assay. Chromatogram E shows the radio-HPLC analysis of the 5min time point of [^{18}F]D4-PBR111 in the rat microsomal assay while Chromatogram F shows the radio-HPLC analysis of the 45min time point of [^{18}F]D4-PBR111 in the Human microsomal assay

The microsomal assay is a non-invasive method for screening the metabolic fate of the two radiotracers during phase 1 metabolism, the main pathway linked to the oxidative cleavage of the propyl chain leading to de-fluorination. From our assay results 7 visible [^{18}F] radio metabolite peaks were identified via radio-HPLC and were given a numerical value based on their polarity on the HPLC chromatogram, metabolite 1 ([^{18}F] fluoride ion) being the most polar metabolite eluting first and metabolite 7 being the most lipophilic and eluting after the intact tracer (Panels E and F). The *in vitro* assays showed that rat microsomes metabolised both [^{18}F]PBR111 and [^{18}F]D4-PBR111 much faster than the human microsomes.

[^{18}F]D4-PBR111 was metabolised slower than [^{18}F]PBR111 in both human and rat microsomes. Interestingly, metabolism of [^{18}F]PBR111 resulted in a much higher percentage of polar metabolites compared to [^{18}F]D4-PBR111. This was observed across both types of microsomes suggesting that the incorporation of the deuterium has significantly reduced the amount of polar metabolites produced by phase 1 metabolism.

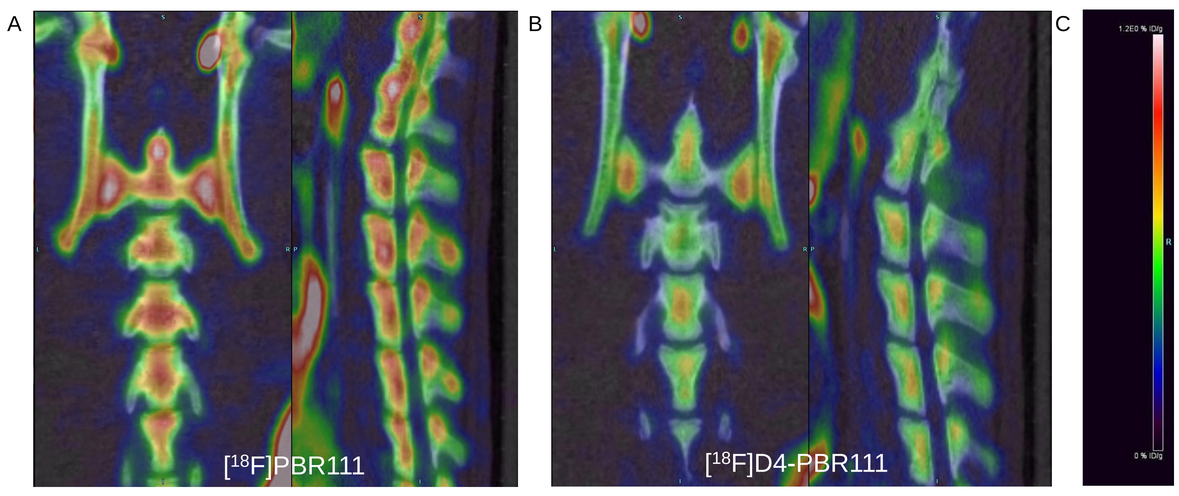
Metabolite analysis of rat plasma using radio-HPLC



HPLC analysis of radioactive components in rat plasma over a 60min time period. Rats were injected with [^{18}F]PBR111 (Panel A) or [^{18}F]D4-PBR111 (Panel B). The total % of radioactivity in the plasma samples consists of 3 major peaks. Peak 1 (◆) contains most polar metabolites including free [^{18}F], Peak 2 (■) consists of the hydrophilic metabolites, Peak 3 (▲) containing the unchanged [^{18}F]PBR111 or [^{18}F]D4-PBR111.

Radio-HPLC analysis of the plasma samples showed the deuterated compound with an increase (36%) in stability over the 60 minute time period, with 21.8% ± 2.7 of the deuterated radiotracer intact at 60 mins compared with the non-deuterated analogue containing only 13.9% ± 4.4 at the same time period. The deuterated analogue also showed a decrease (19%) in the amount of polar metabolite containing the [^{18}F]fluoride with only 67.7% present in the 60 minute plasma sample compared with 83.5% that was observed on the non-deuterated analogue.

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



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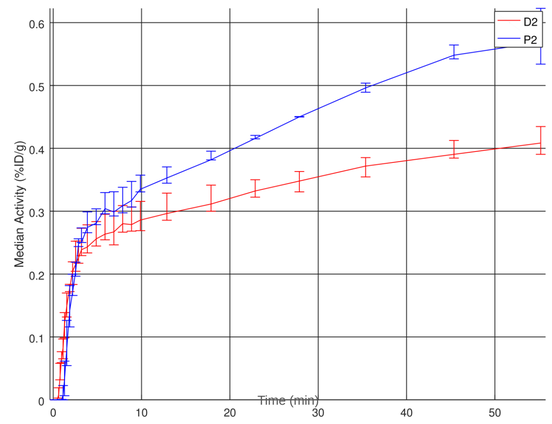

In vivo PET/CT imaging showing the coronal and sagittal planes of the spine of 2 male Sprague Dawley rats. Panel A injected with [^{18}F] PBR111 and Panel B injected with [^{18}F]D4-PBR111 radiotracer. The time frame of the image is 60mins after injection and scaling is 0-1.2%ID/g (Panel C). Panel D represents the time activity curve for median %ID/g in the vertebrae for rats (n=4) injected with [^{18}F] PBR111 (P2) and rats (n=4) injected with [^{18}F]D4-PBR111 (D2)

In vivo PET imaging in rats showed a 42% reduction of the median [^{18}F]D4-PBR111 uptake in bone (vertebrae) compared to non-deuterated [^{18}F] PBR111. This data supports the hypothesis that the introduction of deuterium has significantly reduced the de-fluorination of the PBR111 radiotracer.

Conclusion

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.

References

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