In Vivo/In Vitro Characterisation of a Novel MAO-B Inhibitor Radioligand, Fluorine-18 Labeled Deuterated Fluorodeprenyl (\(^{18}\)F-Fluorodeprenyl-D\(_2\))

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Abstract

The aim of this study was to radiolabel a novel bis-deuterium substituted L-deprenyl analogue (\(^{18}\text{F}-\text{fluorodeprenyl-D}_2\)) with fluorine-18 and to evaluate its potential to visualize and quantify monoamine oxidase B (MAO-B) activity in vivo.

Methods: The precursor compound (5a + 5b) and reference standard (6) were synthesized in multi-step syntheses. Recombinant human MAO-B and MAO-A enzyme preparations were used in order to determine IC50 values. Radiolabeling was accomplished by nucleophilic substitution reaction. Whole hemisphere autoradiography (ARG) was performed with \(^{18}\text{F}-\text{fluorodeprenyl-D}_2\). A positron emission tomography (PET) study was carried out in a cynomolgus monkey. Radiometabolites were measured in monkey plasma using HPLC.

Results: Compound 6 inhibited MAO-B with an IC50 of \(227 \pm 36.8\) nM. Radiolabeling was accomplished with high radiochemical yield, purity and specific radioactivity. The ARG binding density of \(^{18}\text{F}-\text{fluorodeprenyl-D}_2\) was consistent with known MAO-B expression in the human brain. In vivo \(^{18}\text{F}-\text{fluorodeprenyl-D}_2\) showed favorable kinetic properties with relatively fast wash-out from the brain. Regional time activity curves were better described by 2 Tissue Compartment Model. The administration 1 mg/Kg of L-Deprenyl yielded to 70 % inhibition of MAO-B in all regions. Radiometabolite studies demonstrated 20% unchanged radioligand at 120 min post injection. \(^{18}\text{F}-\text{Fluorodeprenyl-D}_2\) showed a less irreversible behavior than previously reported MAO-B radioligands.

Conclusion: The results suggest that \(^{18}\text{F}-\text{fluorodeprenyl-D}_2\) is suitable PET radioligand for visualization of MAO-B activity in human brain.

Key Words: \(^{18}\text{F}-\text{Fluorodeprenyl-D}_2\), monoamine oxidase, monkey, PET.
**Introduction**

Monoamine oxidases (MAO), plays an important role in regulating the chemical neurotransmitters by catalysing oxidative deamination of e.g. monoamine neurotransmitters [1]. MAO is an important therapeutic target for neurological disorders such as Alzheimer’s disease [2], Parkinson’s disease [3] and depression [4]. According to biochemical and pharmacological properties this enzyme exists in two isoforms "MAO Type A" (MAO-A) and "MAO Type B" (MAO-B) [5]. In the human brain MAO-B is the predominant isoform and constitutes up to ~70% of total brain MAO activity [6]. MAO-B selectively oxidizes monoamines such as O-tyramine, phenethylamine and tele-N-methyl histamine and generates hydrogen peroxide which can react to form highly reactive oxygen species [7]. Previously reported increased levels of MAO-B level in Alzheimer’s disease patients might be associated with a progressive increase of oxidative stress [8] and a consequent reactive astrogliosis [9, 10]. As astrocyte activity and, consequently, the activity of the MAO-B system, is up-regulated in neuroinflammatory processes, radiolabeled MAO-B inhibitors may serve as an imaging biomarker in neuroinflammation and neurodegeneration, including Alzheimer’s disease [11].

Positron emission tomography (PET) a high-resolution, sensitive and non-invasive molecular imaging technique has been successfully utilized in visualizing the localization of MAO-B activity for studying neurodegenerative diseases [12]. Several radioligands have been developed in order to study MAO-B activity by PET such as \(^{11}\text{C}\)-pargyline [13], \(^{11}\text{C}\)-deprenyl [12], \(^{11}\text{C}\)-deprenyl-D\(_2\) [2], \(^{11}\text{C}\)-SL25.1188 [14], \(^{11}\text{C}\)-MD230254 [15], \(DL\)-4-\(^{18}\text{F}\)-fluorodeprenyl [16], 6-\(^{18}\text{F}\)-fluoro-N-methyl-N-(prop-2-yn-1-yl)- hexan-1-amine [17], \(^{18}\text{F}\)-Ro 43-0463 [18], \(^{18}\text{F}\)-(S)-3-(6-(3-fluoropropoxy)benzo[d]isoxazol-3-yl)-5-(methoxymethyl)-oxazolidin-2-one [19]. Among those only carbon-11 labeled compounds such as \(^{11}\text{C}\)-deprenyl [12], \(^{11}\text{C}\)-L-deprenyl-D\(_2\) [20] and \(^{11}\text{C}\)-SL25.1188 [21] were demonstrated as useful tracers for assessing MAO-B in human
brain. The short half life of carbon-11 (20.4 min) makes these tracers less suitable for distribution to PET centers not equipped with an on-site cyclotron. So far no successful fluorine-18 labeled PET radioligand has been validated for clinical use. Therefore, there is a high interest in the development of a fluorine-18 labeled MAO-B inhibitor with a longer half-life and a less reversible kinetic behavior as molecular imaging biomarker for the detection of MAO-B activity in brain. Such radioligand might be a useful imaging tool for the detection of activated astrocytes in CNS disorders such as Alzheimer’s disease, Parkinson’s disease and epilepsy. In our recent publications we have reported several fluorine-18 labeled radio tracers such as $^{18}$F-fluorodeprenyl-$\left( N-\left( (2S)-1-^{18}$F-fluoro-3-phenylpropan-2-yl$\right)-N$-methylprop-2-yn-1-amine$\right)$ [22, 23], $^{18}$F-fluororasagiline[24], $(S)-N-(1-^{18}$F-fluoro-3-(furan-2-yl)propan-2-yl$)-N$-methylprop-2-yn-1-amine and $(S)-1-^{18}$F-fluoro-$N,4$-dimethyl-$N$-(prop-2-ynyl)pentan-2-amine[25], $^{18}$F-fluororasagiline-D$_2$[26].

The present work is the continuation of our previous study where we reported in vivo evaluation of MAO-B in cynomolgus monkey brain using $^{18}$F-fluorodeprenyl and also investigated its metabolism[23]. Quantification of MAO-B using $^{18}$F-fluorodeprenyl may not be optimal as its fast irreversible binding to the enzyme renders the distribution of this radioligand in tissue limited by blood flow rather than by the MAO-B enzyme concentration in regions with high MAO-B activity as it was shown for $^{11}$C-deprenyl[20]. Indeed, $^{18}$F-Fluorodeprenyl showed a similar in vivo kinetic behavior as $^{11}$C-deprenyl[27]. It is reported that MAO-B catalyzed bond cleavage of carbon-hydrogen bond of propargyl group at the carbon is the rate limiting step in the retention of radioligand in the brain[28]. The energy required to cleave the C-D bond is higher than that required to cleave the C-H bond and the substitution of hydrogen atoms with deuterium atoms, reduces the rate of cleavage. The aim of the deuteration is to decrease the affinity to
MAO-B based on a reduced cleavage rate of the propargyl moiety and thereby improve its sensitivity.

Therefore, our aims of this project were (i) to prepare the precursor and reference standard and to develop an efficient synthetic method for labeling deuterated fluorodeprenyl with fluorine-18 (ii) to characterize its in vitro MAO-B and MAO-A inhibition based on the rate of kynuramine oxidation (iii) to evaluate the in vitro ARG in post-mortem human brain and (iv) to study the in vivo characteristics by PET measurements in non-human primates.

Materials and Methods

Chemistry

The details experimental procedures for the synthesis of \((1,1-^2\text{H}_2)\text{prop-2-yn-1-ol} \text{ (2) and (1,1-^2\text{H}_2)3-bromoprop-1-yne (3) and (S)-2-(((1,1-^2\text{H}_2)-2-ynyl)(methyl)amino)-3-phenyl propan-1-ol (4) are described in supplementary section.}

Synthesis of \(N-(2\text{-chloro-3-phenylpropyl})-N\text{-methyl-}[1,1-^2\text{H}_2]\text{prop-2-yn-1-amine} \text{ (5a) and (S)-N-(1\text{-chloro-3-phenylpropan-2-yl})-N\text{-methyl-}[1,1-^2\text{H}_2]\text{prop-2-yn-1-amine} \text{ (5b) }

A mixture of 4 (100.0 mg, 0.49 mmol) and triethyl amine (139 µl, 1.0 mmol) in THF (2 ml) was stirred at room temperature for 30 min. To the stirred mixture mesylchloride (68.7 mg, 46.4 µl, 0.60 mmol) was added drop-wise at -7°C and the reaction mixture was stirred at room temperature for additional 30 min. Saturated aqueous Na₂CO₃ solution (1 mL) was added and stirred for further 30 min. The organic layer was partitioned between CH₂Cl₂ (15 ml) and water (15 ml). The organic phase was separated and washed with saturated NaHCO₃ solution (15 ml) and brine (15 ml), dried over MgSO₄ and filtered. The solvent was removed to obtain the crude
product as light yellow oil. The crude product was purified by silica-gel column chromatography (hexane/ethyl acetate 3:1) and final product (70 mg, 64%, 0.32 mmol) was obtained as light yellow oil. The product was analyzed by NMR, HPLC and LC-MS. The final product was a mixture of 5a (Major) and 5b (Minor).

Synthesis of (S)-N-(1-fluoro-3-phenylpropan-2-yl)-N-methyl-[1,1-2H2]prop-2-yn-1-amine] (6)

To the stirred solution of 4 (100 mg, 0.49 mmol) in dichloromethane (5 mL) diethylamino sulfurtrifluoride (132 µl, 1.0 mmol) was added dropwise at -5°C and the reaction mixture was stirred for additional 20 min at the same temperature. Saturated sodium carbonate (2.0 mL) was added to quench unreacted DAST. The organic layer was partitioned between CH2Cl2 (25 ml) and water (15 ml). The organic phase was separated and washed with brine (10 ml) and dried over MgSO4 and filtered. The solvent was removed to obtain the crude product as light yellow oil. The crude product was purified by silica-gel column chromatography (hexane/ether 3:1) and gave the final product (35 mg, 0.17 mmol, 35%). The product was analyzed by NMR, HPLC and LC-MS.

Radiosynthesis of (S)-N-(1-18F-fluoro-3-phenylpropan-2-yl)-N-methyl-[1,1-2H2]prop-2-yn-1-amine] (18F-fluorodeprenyl-D2,7)

Fluorine-18 fluoride (18F-F-) was produced from a GEMS PET trace Cyclotron. A dry complex of 18F-F-/K2CO3/K2.2.2 was synthesized following a previously described method [22]. The precursor (~ 0.02 mmol, ~5mg) in DMSO (dimethyl sulfoxide) (500 µL) was added to the dry complex of 18F-F-/K2CO3/K2.2.2. The closed reaction vessel was heated at 140°C for 10 min. The reaction mixture was cooled to room temperature and diluted with water to a total volume of
2 mL before injecting to the HPLC for purification. A radioactive fraction corresponding to the pure 7 (t<sub>R</sub>=13-14 min) was collected and diluted with water (50 mL). The resulting mixture was loaded on to a pre-conditioned SepPak tC18 plus cartridge. The cartridge was washed with water (10 mL) and the isolated product, was eluted with 1 mL of ethanol in to a sterile vial containing phosphate buffered saline solution (9 mL).

**Determination of MAO Inhibition and In Vitro Autoradiography**

Human recombinant MAO-B and MAO-A enzymes prepared from insect cells were purchased from Sigma. The assays were designed to determine the inhibition of kynuramine oxidation in the presence of the compounds of interest according to Weissbach et al [29]. Details of the experimental procedure are described in supplementary section.

Human brains without pathology were obtained from the Department of Forensic and Insurance Medicine, Semmelweis University, Budapest. The brains had been removed during forensic autopsy (control brains) and were handled in a manner similar to that described previously [30]. Ethical permissions were obtained from the relevant Research Ethics Committee of the respective institutions. Detail experimental procedure is described in supplementary section.

**Radiometabolite Analysis and Plasma Protein Binding**

A reversed phase HPLC (high performance liquid chromatography) method was used for determination of the percentages of radioactivity corresponding to unchanged radioligand and radiometabolites during the course of a PET measurement. Detail procedure is described in supplementary section.
Study Design in non human primates, PET experimental procedure and Quantification

Three male cynomolgus monkeys (Macaca fascicularis) were included in the study. The monkeys (referred to as non-human primates – NHPs) are owned by the Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet. The study was approved by the Animal Research Ethical Committee of the Northern Stockholm Region (Dnr N386/09 and N452/11) Swedish Animal Welfare Agency. Details of the experiment are described in the Supplementary appendix. Altogether five PET measurements of 180 minutes were performed using the high-resolution research tomography (HRRT) (Siemens Molecular Imaging) in three macaques on separate days. Initially a baseline measurement was performed for 3 monkeys. In separate days 2 of the monkeys underwent a second PET measurement after pretreatment with the MAO-B blocker L-Deprenyl 30 minutes before the injection of the radioligand. L-Deprenyl was infused i.v. over 10 min and the selected dose of 1mg/kg was based on previous PET studies in baboon [31]. T1-weighted MR (magnetic resonance) images were acquired for all monkeys with an MRI system GE 1.5 T Signa unit (128 with 1.0 mm slices). Volumes of interest (VOIs) were delineated on co-registered MR/PET images and the delineation was guided by the references of an atlas obtained with 1 rhesus monkey brain. VOIs for caudate, putamen, thalamus, frontal, temporal, parietal and occipital cortex, medial temporal area, cerebellum, and whole brain, were drawn using the software PMOD (PMOD 3.3, PMOD Technologies). The MR image was automatically co-registered and re-sliced to an average PET image using the FUSION tool in the software PMOD. Decay corrected time activity curves for all regions plotted over time and radioactivity were expressed as percentage standardized uptake value (SUV).

All the dynamic PET data were quantified with kinetic modeling with compartmental model analysis with the following 3 methods: 1-TCM with 2 rate constants (K1, k2), 2-TCM with 4 rate constants (K1, k2, k3, and k4) (2TCM 4K) and 2-TCM with 3 rate constants (K1, k2, k3)
The following measures were used to directly and indirectly assess the goodness of fit: Standard Error of Residuals, Akaike Information Criterion and the Schwartz Criterion. The F test was used to assess whether the 2-TCM (4K) provided a significantly better fit than the 2-TCM (3K). Based on this evaluation and the evident reversibility of the tracer we selected as a model of choice the 2TCM (4K) and as outcome measure the total distribution volume ($V_T$). Accordingly we used the revisited Lassen plot[32] method to calculate the amount of inhibition that occurred after the administration of a well known MAO-B blocker.

Results

Chemistry and Radiochemistry

The precursor compounds (5a and 5b) and the reference standard (6) were synthesised by standard organic synthesis from the commercially available starting material propiolic acid (1). The radiolabeling was achieved by nucleophilic substitution of the chloroprecursor mixture of 5a and 5b by $^{18}$F-fluoride in presence of $K_2CO_3$ and $K_2CO_3$ as shown in Figure 2 . The overall radiosynthesis including the fluorination reaction, HPLC purification, SPE purification and radiotracer formulation was completed within 75-80 minutes. The incorporation yield of the fluorination reactions varied from 45% to 55%, and the radiochemical purity was higher than 99%. The identity of the labeled compound was confirmed by co-injection of their corresponding fluorine-19 analogue 6 using analytical HPLC. The radioligand $^{18}$F-fluorodeprenyl-D$_2$ was found to be stable in PBS buffered solution (pH=7.4) for the duration of 120 min. $^{18}$F-fluorodeprenyl-D$_2$ was obtained with a specific radioactivity of $677\pm182$ GBq/µmol.

In vitro Inhibition and Autoradiography
MAO-B and MAO-A inhibitions were determined based on the rate of kynuramine oxidation for the fluorine-19 analogue of fluorodeprenyl-D2 (6) in order to determine its specificity for binding to MAO-B. Compound 6 inhibited the MAO-B activity with an IC$_{50}$ of 227 ± 36.8 nM and the MAO-A activity with an IC$_{50}$ of >50 µM. L-deprenyl and F-deprenyl were used as reference compounds inhibiting MAO-B with an IC$_{50}$ of 13±0.4 and 31±2.2 nM, respectively. Thus, deuteration in compound 6 decreased the IC50 towards MAO-B by ca. 7 times as compared to the nondeuterated fluorodeprenyl in the assay used.

The in vitro binding of $^{18}$F-fluorodeprenyl-D$_2$ was determined in whole hemisphere human brain sections at coronal and horizontal level obtained from deceased subjects with no sign of any brain disorders. The $^{18}$F-fluorodeprenyl-D$_2$ radioligand showed higher selectivity for the MAO-B enzyme in comparison to the MAO-A enzyme as in vitro binding were almost 100 % blocked with the cold ligand L-deprenyl (10 µM), selective inhibitor of the MAO-B enzyme (Figure 3). In contrast, the $^{18}$F-fluorodeprenyl-D$_2$ binding was blocked 12-20% using the cold compound pirlindole (10 µM), selective inhibitor of the MAO-A enzyme (Figure 3). In addition, the $^{18}$F-fluorodeprenyl-D$_2$ baseline binding in whole hemisphere human brain sections showed high in vitro binding in the caudatus, putamen, globus pallidum and thalamus, brain regions with high MAO-B activity and lower radioactivity was measured in the cortex and cerebellum.

**PET measurements in cynomolgus monkey**

The time activity curves (TAC) of $^{18}$F-fluorodeprenyl-D$_2$ uptakes in a cynomolgus monkey brain is shown in Figure 1. $^{18}$F-Fluorodeprenyl-D$_2$ crosses the blood-brain barrier and binds rapidly with a time to peak on average of 4 minutes. At baselines the highest radioactivity concentration expressed with SUV were in the striatum (5.1±1.6), in the thalamus (4.6±1.3) followed by the medial temporal area (4.6±2.5). Overall $^{18}$F-Fluorodeprenyl-D$_2$ showed fast and constant wash-out from the brain in sub-cortical and cortical regions in the baseline scans (Figure 4). A 1-TCM
did not provide a good fitting by visual inspection suggesting the presence of a kinetically
distinguished compartment and was not pursued for further analysis. A 2-TCM 4K provided a
significantly better estimation of the parameters in all baseline scans compared to 2-TCM (3K)
except for the thalamus of one NHP. Administration of L-deprenyl (1.0 mg/kg) decreased 18F-
fluorodeprenyl-D$_2$ binding in all brain regions and determined a clear slope change in the time
activity curves (Figure 4). 2-TCM 4K provided, in the pre-treatment condition, a statistical
improved fitting of the data in one NHP. Overall 4 out of 5 experiments did show a better
estimation of the parameters with 2TCM (4K) providing distribution volumes ($V_T$) estimates that
could be used as outcome measure (Table 1). Thereby MAO-B occupancy of L-Deprenyl
calculated with the revised Lassen Plot was 69% (NHP 2) and 77 % (NHP 3). $V_{ND}$ values
estimated at x-axis intercept in the Lassen plots was 3.7 and 9.8 mL/cm$^3$ (Figure 5).

**Radiometabolite analysis**

The radioactivity in arterial blood samples, plasma and remaining protein pellet after
deproteinization of plasma with acetonitrile were measured by a well counter. The recovery of
radioactivity from plasma into acetonitrile was more than 85%. The plasma obtained from arterial
blood samples taken at various time points following the injection of 18F-fluorodeprenyl-D$_2$ were
analysed by radio-HPLC. All the detected radiometabolites were less lipophilic than the
radioligand. The most lipophilic radiometabolite, eluting with a Rt of 7.9 min closely before the
radioligand Rt 8.6 min, was identified as 18F-fluorometamphatamine-D$_2$ (Figure 6A) by
comparing its Rt to the synthesized reference compound. Approximately 5% of the total
radioactivity originated from 18F-fluorometamphetamine-D$_2$ during the PET scan as shown in
Figure 6B.
The protein binding of 18F-fluorodeprenyl-D2 was measured using ultrafiltration and an average of (33.8 ± 4.1)% of the radioligand was in protein-free form.

**Discussions**

It has been showed that the di-deuteriated derivative of MAO-B inhibitor deprenyl, can be used as a marker for the density of astrocytes in the brain [33, 34]. In the present study we show the potential of 18F-fluorodeprenyl-D2 to visualize MAO-B activity in vivo.

Compound 2 was synthesized by treating propiolic acid with lithium aluminum deuteride. The key parameter of this reaction was to keep the temperature at below -50°C. In the second step, 2 was brominated with PBr₃ to synthesise 3. Compound 4 was synthesised from 3 and (4R,5R)-4-methyl 5-phenyl-3-(prop-2-ynyl)oxazolidin-2-one following a previously described procedure [22]. A mixture of two chlorinated isomers 5a and 5b were formed from 4 upon treatment with mesyl chloride. Fluoride 6 was also synthesized from amino alcohol 4 by fluorination with diethylamino sulfurtrifluoride. The formation of regioisomeric chlorides 5a and 5b can be explained by an intermediate aziridinium ion resulting from an intramolecular nucleophilic substitution attack (SNi) of the free electron pair of the nitrogen which has been previously described elsewhere [35]. Radiolabeling was done by one-step nucleophilic substitution reaction where chloride was substituted by 18F-fluoride. The synthesis resulted in two isomers of fluorine-18 labeled products which were purified by semi-preparative HPLC column. High in vitro 18F-fluorodeprenyl-D2 binding was measured in several brain regions with high MAO-B activity and L-deprenyl (10 μM) successfully displaced all 18F-fluorodeprenyl-D2 binding. In vivo, in NHP, 18F-fluorodeprenyl-D2 showed favorable kinetic properties with relatively steady wash-out from the brain in most of the experiments and conditions. The
deuteration seemed, in vivo, to lead to a further reduction of the covalent binding of the tracer to the enzyme resulting in a less irreversible behavior even if it is compared, in the same experimental condition and NHP with \(^{11}\text{C}\) Deprenyl-D2 kinetic (Figure.7 and supplementary section for details). In the present study a more reversible kinetic of \(^{18}\text{F}\)-fluorodeprenyl-D2 was confirmed by models comparison. The 2 tissue compartments model suitable for irreversible tracers (3 rate constants) did not provide the best performances in term of fitting in most of the experiments hence the 2 tissue compartments model (4 constant rates) was the most appropriate model to describe the brain kinetic of \(^{18}\text{F}\)-fluorodeprenyl-D2. Consequently the estimation of total distribution volume (VT) was considered as a more reliable outcome measure to evaluate the availability of MAO-B in NHP. Moreover the estimates of \(V_{ND}\) obtained from the revised Lassen Plot allowed to measure the level of specific binding which in the case of a high density MAO-B region such as the thalamus (VT values on average 34.91 mL/cm\(^3\)) was on average quite high (79\%). The calculated occupancy after pre-treatment with Deprenyl (1mg/Kg) was higher (77\% vs 61.7\%) than a previousestimation obtained with \(^{11}\text{C}\)-deprenyl-D2 (see table 3 in supplementary section).

It is reported that the two main radiometabolites of \(^{11}\text{C}\)-deprenyl are \(^{11}\text{C}\)-CO\(_2\) and \(^{11}\text{C}\)-metamphetamine [36] and at 30 min post injection 30\% of the total radiometabolites found in baboon plasma was in the form of \(^{11}\text{C}\)-CO\(_2\). In the case of \(^{18}\text{F}\)-fluorodeprenyl-D\(_2\) there is no possibility of having radiolabeled CO\(_2\) but more likely to have \(^{18}\text{F}\)-fluorometamphetamine-D\(_2\). The potential specific contribution of one of the most lipophilic radiometabolite is questionable. Indeed one of the identified radiometabolite, \(^{18}\text{F}\)-fluorometamphetamine-D\(_2\), was observed on average < 5\% and in some NHP was not separated on the HPLC because of its low amount. A structurally similar radiotracer has been shown to enter in human brain and bind primarily to the monoamine transporters [37].
The presence of such amount of $^{18}$F-fluorometamphetamine-D$_2$ in the plasma might contribute to a small increase of non specific binding of $^{18}$F-fluorodeprenyl-D$_2$ in the brain. Future challenges would include optimization of the HPLC conditions to achieve a better separation of radiometabolites in plasma and to investigate BBB penetrating radiometabolites by using mouse models.

**Conclusion**

In the present work an efficient synthesis strategy for a $^{18}$F-labeled deuterium substituted fluoro-analog of L-deprenyl was established yielding the target compound. *In vitro* MAO inhibition demonstrated a moderate binding affinity to recombinant MAO-B. *In vitro* ARG demonstrated the selective binding to MAO-B containing brain regions, e.g. striatum. *In vivo* characteristics in cynomolgus monkey showed a moderate brain uptake in known MAO-B rich regions. The deuterated analogue of $^{18}$F-fluorodeprenyl is more stable in monkey blood plasma if compared to the nondeuterated analogue. In addition, the deuteration reduced the rate of radioligand trapping in monkey brain leading to improved sensitivity. PET studies confirmed by the kinetic analysis performed in non human primates. A faster kinetic and the fluorine labeling are possible advantages compared to other previously developed radioligands for the MAO-B imaging such as $^{11}$C-D$_2$-Deprenyl. These results together suggest that $^{18}$F-fluorodeprenyl-D$_2$ is an improved PET radioligand for visualization of MAO-B activity in human brain.

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References


Figure 1. Structures of $^{11}$C-deprenyl, 18F-Fluorodeprenyl and 18F-fluorodeprenyl-D$_2$ (7)
Figure 2. Synthesis of precursor (5a+5b), reference standard (6) and radiolabeling of $^{18}$F-Fluorodeprenyl-D$_2$, (7).

**Conditions:** a: LAD; b: PBr$_3$; c: K$_2$CO$_3$/(1,1-$^2$H$_2$)-1-bromoprop-2-yne; d: pyridine/mesyl chloride; e: DAST; f: K$^{18}$F, K-2,2,2/DMSO/ K$_2$CO$_3$
Figure 3. Autoradiograms of coronal slices in whole hemisphere human brain with 18F-fluorodeprenyl-D<sub>2</sub> (7) at baseline condition and during incubation with pirlindole (10 µM) and L-deprenyl (10 µM).
Figure 4. Representative fused PET/MR images of 18F-fluorodeprenyl-D$_2$ (7) and relative SUV time activity curves (TACs) in the two experimental conditions (baseline) and pretreatment (pretreatment) at the level of the thalamus in one NHP.
Figure 5. Baseline data are used along with the occupancy plot to derive occupancy estimates. Data are taken from the two NHP (2-3) that performed both baseline and the pretreatment study with L-Deprenyl 1mg/kg. In both graphs each data point represents the regions of interest included in the regression analysis.
Figure 6. A. Radiochromatogram of plasma taken 30 min after injection of the radioligand. B. The *in vivo* metabolism of 18F-fluorodeprenyl-D$_2$ (7) is shown as the relative plasma composition of parent compound and the identified radiometabolite 18F-flurometamphetamine-D$_2$ in total plasma radioactivity during 180 min of PET scan. The values are average of measurements performed in three cynomolgus monkeys.
Figure 7. Head to head comparison of two baseline scans performed in the same experimental condition in the same NHP (3) with $^{11}$C-Deprenyl-D$_2$ (A) and $^{18}$F-fluoroDeprenyl-D$_2$ (B).
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<td>19.80</td>
<td>9.73</td>
<td>20.35</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>18.63</td>
<td>8.61</td>
<td>15.52</td>
<td>13.20</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>19.10</td>
<td>6.40</td>
<td>11.04</td>
<td>8.60</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** Total Distribution Values Calculated with 2-TCM (4K) in both the experimental conditions.
In Vivo/In Vitro Characterisation of a Novel MAO-B Inhibitor Radioligand, Fluorine-18 Labeled Deuterated Fluorodeprenyl (\textsuperscript{18}F-Fluorodeprenyl-D\textsubscript{2})

Sangram Nag, Patrik Fazio, Lutz Lehmann, Georg Kettschau, Tobias Heinrich, Andrea Thiele, Marie Svedberg, Nahid Amini, Samira Leesch, Ana Catafau, Jonas Hannestad, Andrea Varrone and Christer Halldin

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