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Radiosynthesis of [^{18}F]AV1451 in pharmaceutical conditions and its biological characteristics

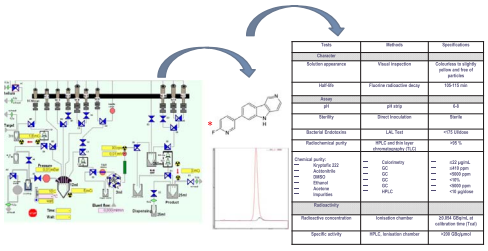
Anne-Sophie Salabert[a,b,*], Charlotte Fontan[b], Caroline Fonta[c], Mathieu Alonso[b], Najat Loukh[a,d], Marie Bernadette Delisle[a,d], Mathieu Tafani[a,b], Pierre Payoux[a,e]

[a] "Toulouse Neuro-imaging Centre" Research Unit (UMR 1214) INSERM, Toulouse, France
[b] Radiopharmacy Department, Toulouse University Hospital, Toulouse, France
[c] Brain & Cognition Research Centre (CERCO UMR 5549), Toulouse, France
[d] Neuropathology, Toulouse University Hospital, Toulouse, France
[e] Department of Nuclear Medicine, Toulouse University Hospital, Toulouse, France

HIGHLIGHTS

- We performed radiofluorination with a pyridyl trialkyl substituted ammonium salt derivative.
- Pharmaceutical quality of [^{18}F]AV1451 was determined.
- We observed plasma protein binding of [^{18}F]AV1451.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we describe the radiosynthesis of [^{18}F]AV1451 in terms of its pharmaceutical quality and characterise its physical and biological properties. We performed an in vitro serum stability study in fresh human plasma and a plasma protein binding study. The radiochemical yield was 24% (decay corrected), and the product met all regulatory quality requirements. We found that this compound is stable in fresh human plasma and binds tightly to plasma proteins, especially lipoproteins.

1. Introduction

Alzheimer's disease (AD) is the most frequently presenting neurodegenerative diseases, affecting nearly 35 million people worldwide (James et al., 2015; Ariza et al., 2015). The diagnosis of AD can only be confirmed post mortem on the basis of the evidence of specific brain lesions. These neuropathological lesions are a combination of β-amyloid peptide deposits (APs) within plaques and hyperphosphorylated tau (tau-PHF) in neurofibrillary tangles (NFT), in neuropil threads and around amyloid deposits in neuritic plaques (Braak and Braak, 1995; Delacourte, 2006; Hardy and Selkoe, 2002; Dietmar R. Thal et al., 2002). However, over the last 10 years, biomarkers (including research on amyloid, tau and phospho-tau in the CSF) have become useful in the diagnosis of this pathology (Jack et al., 2011; McKhann et al., 2011).

Molecular imaging with positron emission tomography (PET) or single-photon emission computed tomography (SPECT) has demonstrated its ability to be a complementary technique for exploring neuronal and metabolic changes in AD. [^{18}F]fluoro-D-glucose can be used

* Correspondence to: UMR 1214 Inserm TONIC (Toulouse Neuro-imaging Centre), Université Toulouse III - Paul Sabatier, CHU PURPAN - Pavillon BAUDOT, Place du Dr Joseph Baylac, 31024 Toulouse cedex 3, France.
E-mail addresses: anne-sophie.salabert@inserm.fr (A.-S. Salabert), Fontan.Charlotte@iuct-oncopole.fr (C. Fontan), caroline.fonta@cerco.ups-tlse.fr (C. Fonta), alonso.m@chu-toulouse.fr (M. Alonso), loukh.n@chu-toulouse.fr (N. Loukh), delisle.b@chu-toulouse.fr (M.B. Delisle), tafani.m@chu-toulouse.fr (M. Tafani), payoux.p@chu-toulouse.fr (P. Payoux).

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to highlight decreased glucose metabolism in hippocampal and temporoparietal cortical areas in patients, while the development of radiotracers that bind to APs has allowed for the non-invasive in vivo assessment of AD brain lesions. Several AP-selective PET radioligands have been assessed in recent years, including [^{11}C]PIB (Agdeppa et al., 2001), [^{18}F]-florbetapir (Henriksen et al., 2008), [^{18}F]-Florbetaben (Lister-James et al., 2011) and [^{18}F]-Flutemetamol (Dietmar Rudolf Thal et al., 2015).

These amyloid tracers are very useful to describe brain amyloid peptide distribution. However, amyloid deposits are known to occur without any detectable clinical symptoms.

With regard tau-PHF, Braak and Braak (Braak and Braak, 1995) have shown that the density and distribution of NFT depend on the stage of the disease, following a progressive course that ends with a major invasion of the cortex. They identified six stages correlated with cognitive dysfunction. Molecular imaging using a tracer targeting tau-PHF is a new tool for assessing the process of neurodegeneration in AD as the level of this abnormal tau is correlated with the severity of the disease (Buée et al., 2000; Delacourte, 2006; Jicha et al., 2012; Shah and Catafau, 2014).

Several PET radiopharmaceuticals have recently been developed to target abnormal tau conformations. To date, seven imaging agents have been described and are regarded as very promising TAU radioligands (Table 1).

[^{18}F]AV1451 is the same compound as [^{18}F]T807 but is referred to as [^{18}F]AV1451 throughout this publication. This compound belongs to Lilly pharmaceutical®.

[^{18}F]AV1451 binds tau-PHF with nanomolar affinity and is 25 times more selective for the tau-PHF than for Aß, proving its high specificity (Chien et al., 2013).

PET studies have shown a rapid penetration into the brain and rapid clearance in mice (Xia et al., 2013). Kidney elimination was a significant clearance pathway. Four metabolites were detected, all with a shorter retention time (RT) than [^{18}F]AV1451. In mice, 60% of [^{18}F]AV1451 remained intact 30 min after radiotracer injection (Declercq et al., 2016). To predict possible metabolism in humans, the tracer was incubated with human liver microsomes and was found to be much more stable than when incubated with mice liver microsomes (Xia et al., 2013). However, in vitro plasma stability of [^{18}F]AV1451 has not been tested on human plasma, and inter-species variability could exist. Plasma degradation could lead to the presence of metabolites causing a nonspecific signal that can damage the specificity and quality of PET imaging. To conduct quantification studies, it is important to have free fraction plasma data. This may not be a problem as long as the free fraction is constant throughout the experimental setting and the correction can be disregarded. However, it becomes a serious problem when the free fraction of the ligand in plasma is subject to change in pathological conditions (Turkheimer et al., 2015).

This article includes the description of [^{18}F]AV1451 preparation using Raytest® fluorination module and quality control of this experimental drug for human use (pre-validation run for clinical trials). In the second part, to have a better knowledge of [^{18}F]AV1451's biological properties, the lipophilicity, plasma stability and plasma protein binding of this radioligand were evaluated.

2. Materials and methods

2.1. Radiochemical synthesis

2.1.1. Reagents and apparatus

We obtained a preconditioned Sep-Pak® Light QMA cartridge with carbonate as counter ions and elution reagent (600 µL with 22 mg cryptand 222, 7 mg potassium carbonate, 300 µL acetonitrile, 300 µL water for injection) from Advanced Biomedical Compounds (ABX GmbH, Heinrich-Glaeser-Strasse 10, 14 01454 Radeberg, Germany). C_{18} Sep-Pak® Plus cartridges were obtained from Waters (Milford, MA, USA). These cartridges were then conditioned with 5 mL ethanol and 5 mL sterile water. Radioactivity was determined using a dose calibrator (Capintec® CRC-25). Automated synthesis was carried out in a Raytest® SynChrom R & D unit.

High-performance liquid chromatography (HPLC) for [^{18}F]AV1451 purification was carried out in a Raytest® synthesis module with a built-in HPLC system featuring a semi-preparative column and a C_{18} Sep-Pak precolumn. The semi-preparative HPLC was equipped with a UV detector (Knauer K-200 miniaturised UV detector) and a radioactivity detector (Raytest® Ramona star). Semi-preparative HPLC purification was conducted in a reverse column (Zorbax Eclipse XDB-C18, 9.4 × 250 mm, 5 µm). HPLC data acquisition and analysis were performed using Gina Star software.

For quality control, HPLC analysis was conducted on a modular HPLC system (Bischoff pump 3350) equipped with a reversed-phase analytic C_{18} column (4.6 × 150 mm, Luna® Phenomenex). The AV1622 (Xiong et al., 2015) and [^{19}F]AV1451 reference standards were obtained from Avid Radiopharmaceuticals (3711 Market Street, 7th Floor Philadelphia, PA 19104 phone: 215-298-0700, fax: 413-826-0416)

This HPLC system was coupled with two detectors: Detector 1 measured the radioactivity (NaI crystal, Raytest GABI detector) and Detector 2 measured the UV signal at 270 nm (ICS Lambda 1010 UV–VIS Detector). Acquisition and data analyses were performed using AQUIS® ICS software.

2.1.2. Automated synthesis of [^{18}F]AV1451

2.1.2.1. Manufacturing process. [^{18}F]$^-$ fluoride is obtained through the nuclear reaction ^{18}O (p, n) ^{18}F by the irradiation of > 97% ^{18}O-enriched water (Eurisotop®) target (2.1 mL) with a 10-MeV proton beam in an IBA 10/5 cyclotron. We typically produce on average 25 GBq per cyclotron beam for these experiments. The typical condition for irradiation was a single beam at 40 µA for 60 min. One synthesis module [^{18}F] is collected in a Sep-Pak QMA cartridge, and $H_2{}^{18}O$ is collected for recycling. A total of 600 µL eluent-reagent solution is passed through the Sep-Pak QMA cartridge, from which the trapped [^{18}F] is sent to the reaction vessel. The solvent is evaporated under helium blanketing at 115 °C. After complete removal of the solvent by azeotropic drying, the precursor AV1622 (2 mg), diluted with DMSO (1.5 mL; Dimethyl sulphoxide dried Merck 1.02931.0161), is added to the reactor vessel. The solution is heated at 110 °C for 5 min to perform the nucleophilic substitution (Fig. 1). To remove protecting groups, hydrolysis is performed with 3 N HCl solution (0.9 mL; Merck), and the mixture is heated at 100 °C for 5 min. The reaction mixture is then

Table 1
Characteristics of PHF-tau [^{18}F] radioligands: Affinity (represented by dissociation constant (Kd)), lipophilicity (represented by octanol-water partition coefficient) and information on their use (preclinical and clinical).

Compound	Kd for PHF-tau (nM)	Lipophilicity	Information
[^{18}F] FDDNP	65	3.92[16]	Lack of specificity: Kd for Aβ= 5 nM [17]
[^{18}F] THK-523	86	2.40 [18]	Lack of specificity: Kd for Aβ = 30 nM [17]
[^{18}F] THK5105	2.63	3.03 [18]	Tested on AD patients [19]
[^{18}F] THK5117	5.19	2.32 [18]	Tested on AD patients[20]
[^{18}F] AV1451 also called ([^{18}F] T-807)	14.6	1.67 [21]	Clinical trials (AD, Traumatic brain injury, etc.)
[^{11}C] PBB3	100	3.92 [22]	Lack of specificity for NFT
[^{11}C] N-methyl-lansoprazole	0.7	2.18 [23]	No fluorine derivative yet