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Technical note

A simple and fast method for the preparation of n.c.a. 2-[^{18}F]F-A85380 for human use

J. Schmaljohann[a,b,*], D. Gündisch[c], M. Minnerop[a], A. Joe[b], J. Bucerius[b], C. Dittmar[e], F. Jessen[d], S. Guhlke[b], U. Wüllner[a]

[a]*Department of Neurology, University of Bonn, Germany*
[b]*Department of Nuclear Medicine, University of Bonn, Germany*
[c]*Department of Pharmaceutical Chemistry, University of Bonn, Germany*
[d]*Department of Psychiatry, University of Bonn, Germany*
[e]*MC Pharma GmbH, Bonn, Germany*

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Abstract

2-[^{18}F]F-A85380 is the first subtype selective PET-radiotracer to visualize the distribution of $\alpha_4\beta_2$ nicotinic acetylcholine receptors in human brain in vivo. We investigated a fast and safe automated production of 2-[^{18}F]F-A85380 by purification of the BOC-protected intermediate product with a combination of solid phase extraction cartridges. After deprotection, adjustment of the pH and sterile filtration n.c.a. 2-[^{18}F]F-A85380 was applicable for the use in human studies with a high specific activity and an overall radiochemical yield of 55% in 35 minutes.

Keywords: Nicotinic acetylcholine receptor; Fluor-18; 2-^{18}F-A85380; PET

1. Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs), the prevailing cholinergic receptors in the central nervous system, are assembled from at least 11 distinct subunits, $\alpha_2-\alpha_9/\alpha_{10}$ and $\beta_2-\beta_4$. Although nAChRs contribute to a variety of brain functions, relatively few data are available on their physiological functions and their potential role in neurological diseases (Holladay et al., 1997; Peng et al., 2004). A reduction of different nAChR subtypes has been reported in post-mortem brains of patients with Alzheimer's disease (AD), Parkinson's disease (PD) and dementia with Lewy bodies (DLB) (Whitehouse et al., 1986). Radioligand binding studies and analyses of protein expression in post mortem brain tissue suggested that α_4 is the most susceptible nAChR subunit in AD (Wevers and Schroder 1999; Martin-Ruiz et al., 1999). 2-[^{18}F]F-A85380, a radioligand with high affinity to subtype $\alpha_4\beta_2$ and a high subtype selectivity to β_2 containing nAChRs (Koren et al., 1998; Zoli et al., 1998), is the first subtype selective PET-radiotracer to visualize the distribution of $\alpha_4\beta_2$ nAChR in human brain in vivo (Kimes et al., 2003; Bottlaender et al., 2003; Schmaljohann et al., 2004a).

*Corresponding author. Jörn Schmaljohann, Department of Neurology, University of Bonn, 53105 Bonn, Germany. Tel.: +49 228 2879859; fax: +49 228 2879096.
E-mail address: joern.schmaljohann@ukb.uni-bonn.de (J. Schmaljohann).

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We investigated a fast and safe automated production of 2-[^{18}F]F-A85380 applicable for the use in human studies.

2. Experimental

2.1. General

Chemicals and solvents (analytical grade) were purchased from commercial sources and used without further purification. The precursor 1 and the reference substance 3 (2-Fluoro-A85380) were synthesized according to the literature (Dolle et al., 1998; Dolle et al., 1999) as well as obtained from ABX advanced biochemical compounds (Radeberg, Germany).

2.2. Radiochemistry

The aqueous [^{18}F]fluoride solution (3–30 GBq) was dried in the presence of 1 ml acetonitrile, 25 mg (66 μmol) Kryptofix®K_{222} and 2.8 mg (20 μmol) K_2CO_3. 1.0–2.0 mg (2.1–4.3 μmol) precursor (2-trimethylammonium-3-(1-tert.-butoxycarbonyl-2(S)-azetidinylmethoxy)-pyridine trifluoromethanesulfonate 1) solved in 1 ml DMSO were added and the nucleophilic substitution was performed at 135 °C for 5 min. After cooling to room temperature, the reaction mixtures were diluted with 4 ml water and passed through a combination of solid phase extraction cartridges consisting of ICH (Alltech, previously conditioned with 10 ml water), Sep Pak QMA (Waters, previously conditioned with 10 ml water) and Sep Pak RP18 (Waters, previously conditioned with 10 ml ethanol followed by 10 ml water). The cartridges were washed with 2 ml water and the intermediate product [^{18}F]2 was eluted from the C18-cartridge with 1 ml ethanol into a second reaction vessel. Cleavage of the BOC-group by adding 1 ml 0.2 M hydrochloric acid and simultaneous evaporation of ethanol was achieved in 3 min at 100 °C. After adding 4 ml phosphate buffer (NaH_2PO_4 0.21 M, Na_2HPO_4 5.5 mM, $NaHCO_3$ 0.06 mM; pH = 6.35) to adjust the pH the product [^{18}F]3 was filtrated over a sterile filter (0.2 μm) and collected in a sterile vial. For quality control of the product, an aliquot was analysed by HPLC (k' of 3 = 4.75) using a RP18-column (LiChrosorb RP18 250 × 4 mm, solvent: TEAP pH 2.5 (triethylamin 0.11 M, H_3PO_4 0.15 M), 1 ml/min flow), an UV-detector (Sykam, 254 nm) and a NaI crystal radioactivity detector. TLCs were run on silicagel 60 plates (Machery-Nagel Sil G, Düren Germany) with acetonitrile/water (950/50) as solvent (Rf: [^{18}F]F^- = 0.0, [^{18}F]3 = 0.15, [^{18}F]2 = 0.9) and analyzed using a phosphor imager (Fuji BAS 1800). The detection of further impurities (solvents, Kryptofix®K_{222}) was accord with the standard procedures of FDG quality control.

3. Results and Discussion

Based on the prior published radiosynthesis on n.c.a. 2-[^{18}F]F-A85830 (3) (Dolle et al., 1999; Hori et al., 1996; Schmaljohann et al., 2004b) we developed an automated production for studies in human using a fast and safe solid phase extraction purification. The radiosynthesis of n.c.a. 3 was established with 1 as precursor for the aromatic nucleophilic substitution followed by cleavage of the BOC-group with hydrochloric acid (Fig. 1).

The activated [^{18}F]K_{222}KF-complex was prepared according to standard procedure. The optimized reaction conditions of the nucleophilic substitution (Schmaljohann et al., 2004b) had been adjusted to the automated synthesis module resulting in a radiochemical yield (rcy) for 2 of 70–75% (1 mg 1) and 85–90% (2 mg 1), respectively (determined after nucleophilic substitution by TLC). The BOC-protected product [^{18}F]2 was purified by solid phase extraction with a combination of a cation exchange- an anion exchange- and a reverse phase-cartridge. Using a strong cation exchanger ensured a nearly quantitative removal of the K_{222}-complexes and unreacted precursor 1. But also 5–10% of the initial activity was fixed on the ICH-cartridge after solid phase extraction purification probably resulting from a partially cleavage of the BOC-group on the strong acidic cation exchanger. This small loss of product can be accepted for a higher safety, no impurity of Kryptofix®K_{222} and 1 in the final product, which is a concern for the injection in human. Unreacted [^{18}F]fluoride was trapped on the anion

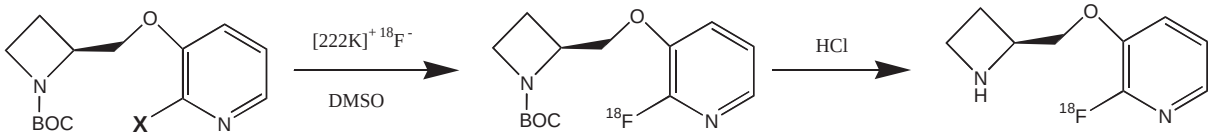

Fig. 1. Radiosynthesis of n.c.a. 2-[^{18}F]Fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine.

exchange-cartridge and the intermediate product [^{18}F]2 was concentrated on the reverse phase-cartridge. Pure [^{18}F]2 could be eluted with ethanol and the BOC-group was nearly quantitatively cleaved with hydrochloric acid during simultaneous evaporation of ethanol. After adjustment of the pH and sterile filtration n.c.a. [^{18}F]3 was ready for injection. Overall radiochemical yield, with respect to initial [^{18}F]fluoride ion radioactivity, was 52–57% in a total synthesis time of 35 min (including drying of the [^{18}F]fluoride). In addition to the loss of radioactivity in the reaction vessels and tubing, an unknown side-product (5%) was observed during the fluoride exchange reaction.

In summary, a routine production of up to 12.5 GBq 2-[^{18}F]F-A85380 with sufficient uncorrected yields of 43–48% was established by a simple and fast solid phase extraction purification, avoiding more complex and fault-prone HPLC-equipment. The purity of the product 3 was suitable for injection in humans, with (1) a specific activity of >300 GBq/μmol (>8.1 Ci/μmol) at EOS, (2) a radiochemical purity of $>95\%$ and (3) no remarkable macroscopic impurities.

References

Bottlaender, M., Valette, H., Roumenov, D., Dolle, F., Coulon, C., Ottavini, M., Hinnen, F., Ricard, M., 2003. Biodistribution and radiation dosimetry of 18F-fluoro-A-85380 in healthy volunteers. J. Nucl. Med. 44 (4), 596–601.

Dolle, F., Valette, H., Bottlaender, M., Hinnen, F., Vaufrey, F., Guenther, I., Crouzel, C., 1998. Synthesis of 2-[^{18}F]Fluoro-3-[2(S)-azetidinylmethoxy]pyridine, a Highly Potent Radioligand for in Vivo Imaging Central Nicotinic Acetylcholine Receptors. J. Labeled Compd. Radiopharm. 41, 451–463.

Dolle, F., Dolci, L., Valette, H., Hinnen, F., Vaufrey, F., Guenther, I., Fuseau, C., Coulon, C., Bottlaender, M., Crouzel, C., 1999. Synthesis and Nicotinic Acetylcholine Receptor in Vivo Binding Properties of 2-Fluoro-3-[2(S)-azetidinylmethoxy]pyridine: a new positron emission tomography ligand for nicotinic receptors. J. Med. Chem. 42, 2251–2259.

Holladay, M.W., Dart, M.J., Lynch, J.K., 1997. Neuronal nicotinic acetylcholine receptors as targets for drug discovery. J. Med. Chem. 40 (26), 4169–4194.

Horti, A.G., Ravert, H.T., London, E.D., Dannals, R.F., 1996. Synthesis of a radiotracer for studying nicotinic acetylcholine receptors: (+/−)-exo-2-(2-[^{18}F]fluoro-5-pyridyl)-7-azabicyclo[2.2.1]heptane. J. Labeled Compd. Radiopharm. 38, 355–365.

Kimes, A.S., Horti, A.G., London, E.D., Chefer, S.I., Contoreggi, C., Ernst, M., Friello, P., Koren, A.O., Kurian, V., Matochik, J.A., Pavlova, O., Vaupel, D.B., Mukhin, A.G., 2003. 2-[^{18}F]F-A-85380: PET imaging of brain nicotinic acetylcholine receptors and whole body distribution in humans. FASEB J 17 (10), 1331–1333.

Koren, A.O., Horti, A.G., Mukhin, A.G., Gündisch, D., Kimes, A.S., Dannals, R.F., London, E.D., 1998. 2-, 5-, and 6-Halo-3-(2(S)-azentinylmethoxy)pyridines: synthesis, affinity for nicotinic acetylcholine receptors, and molecular modeling. J. Med. Chem. 41, 3690–3698.

Martin-Ruiz, C.M., Court, J.A., Molnar, E., Lee, M., Gotti, C., Mamalaki, A., Tsouloufis, T., Tzartos, S., Ballare, C., Perry, R.H., Perry, E.K., 1999. Alpha4 but not alpha3 and alpha7 nicotinic acetylcholine receptor subunits are lost from the temporal cortex in Alzheimer's disease. J. Neurochem. 73 (4), 1635–1640.

Peng, H., Ferris, R.L., Matthews, T., Hiel, H., Lopez-Albaitero, A., Lustig, L.R., 2004. Characterization of the human nicotinic acetylcholine receptor subunit alpha (alpha) 9 (CHRNA9) and alpha (alpha) 10 (CHRNA10) in lymphocytes. Life Sci 76 (3), 263–280.

Schmaljohann, J., Gündisch, D., Joe, A., Minnerop, M., Jessen, F., Schütz, C., Reinhardt, M., Maguire, R.P., Klockgether, T., Biersack, H.-J., Wüllner, U., 2004a. PET imaging of $\alpha4\beta2$ nicotinic acetylcholine receptors in Alzheimer's and Parkinson's disease with 2-[^{18}F]A-85380. Neuroreport, submitted.

Schmaljohann, J., Minnerop, M., Karwath, P., Gündisch, D., Falkai, P., Guhlke, S., Wüllner, U., 2004b. Imaging of central nAChReceptors with 2-[^{18}F]F-A85380: optimized synthesis and in vitro evaluation in Alzheimer's disease. Appl. Rad. Iso. 61 (6), 1235–1240.

Wevers, A., Schroder, H., 1999. Nicotinic Acetylcholine Receptors in Alzheimer's Disease. J. Alzheimer's Dis. 1 (4–5), 207–219.

Whitehouse, P.J., Martino, A.M., Antuono, P.G., Lowenstein, P.R., Coyle, J.T., Price, D.L., Kellar, K.J., 1986. Nicotinic acetylcholine binding sites in Alzheimer's disease. Brain Res 371, 146–151.

Zoli, M., Lena, C., Picciotto, M.R., Changeux, J.P., 1998. Identification of four classes of brain nicotinic receptors using beta2 mutant mice. J. Neurosci. 18 (12), 4461–4472.