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Research Article

Synthesis of ^{18}F labelled FK960, a candidate anti-dementia drug, and PET studies in conscious monkeys

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Summary

The present study demonstrated the synthesis and *in vivo* study of ^{18}F-labeled *N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide (FK960) which is a novel anti-dementia drug candidate. [^{18}F]FK960 was prepared by a one-pot, three reaction sequence, using nucleophilic fluorination, with an automated synthetic apparatus using either ethyl-4-trimethylammonium triflate (**1a**) or ethyl-4-nitrobenzoate (**1b**) as the precursor for labeling. Though **1a** gave a higher yield, the specific activity was 50–100 fold higher with **1b**. The radiochemical yield of [^{18}F]FK960 was 7–15% (EOB) and the specific activity ranged from 2.0–60.2 GBq/μmol depending on the amount of F-18 used. The synthesis time was 2.2–2.9 h. The obtained [^{18}F]FK960 was injected into 3 conscious monkeys (100–120 MBq/kg body weight), and distribution images and pharmacokinetic data for [^{18}F]FK960 showed similar uptake in different brain regions and 3-fold higher levels of [^{18}F]FK960 in blood relative to brain. Copyright © 2002 John Wiley & Sons, Ltd.

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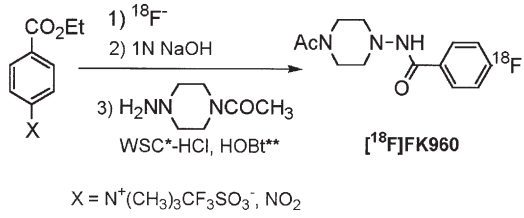

Introduction

N-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide (FK960) is a novel anti-dementia drug candidate that has displayed improved memory impairment in a variety of animal models.[1,2] FK960 is a somatostatin releaser[3,4] and it is well known that somatostatin releases acetylcholine.[5] Therefore, FK960 indirectly activates the release of acetylcholine, and shows efficacy in animal models of scopolamine-induced amnesia[1,2] and neuronal dysfunction.[6]

As described above, the mechanism and pharmacological efficacy of FK960 are becoming clear, however in general, a suitable dose setting is important in order to succeed in clinical trials. Positron emission tomography (PET) can provide important information about the pharmacokinetics of a compound, especially the permeability of the blood-brain barrier both in animals and humans. In order to perform a PET study, labelling of the compound is essential. Because FK960 contains a benzoylamide with a F-atom in the para position, which makes the compound suitable for labelling with ^{18}F, the present study demonstrates the development of a rapid synthesis of ^{18}F labelled FK960 using nucleophilic fluorination with a fully automated synthetic apparatus, and the distribution study of ^{18}F labelled FK960 in conscious monkey brain using PET.

Results and discussion

Chemistry

We first considered the straightforward synthesis shown in Figure 1. We attempted a cold synthesis of [^{18}F]FK960 by nucleophilic substitution reactions of compounds **1** under various conditions. However, the reaction did not proceed due to lack of reactivity of the aromatic ring.

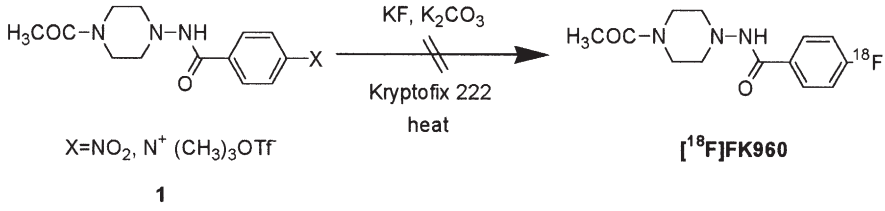

Figure 1. Attempted preparation of [^{18}F]FK960 in one step from compounds 1

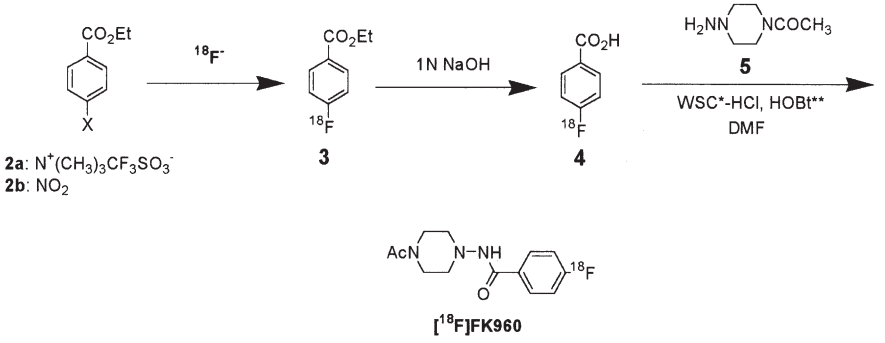

Figure 2. Synthetic route for [^{18}F]FK960. *WSC: 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide. **HOBt: *N*-hydroxybenzotriazole

These results led us to a stepwise route for the synthesis of [^{18}F]FK960 involving nucleophilic substitution on an aromatic ring as the key fluorination reaction, as shown in Figure 2. An ethyl benzoate derivative was chosen as the starting material because hydrolysis could be performed without cleavage of the F-atom. The fluorination conditions were examined based upon reference to various articles.[7–9] For the amidation of 4-fluorobenzoic acid, the reaction mixture was neutralized with 1N-HCl and the solution containing 4-fluorobenzoic acid was evaporated. The evaporation was performed to prevent reaction of methyl sulfoxide (DMSO) with the amidation agent, WSC (ethyl *N,N*-dimethylaminopropyl carbodiimide mono hydrochloride), and to increase the concentration of the reagents. The neutralization before the amidation was critical, because the pH was shown to be the most important parameter in studies of cold synthesis. It was confirmed in our experiments that the amidation reaction proceeds in the pH 3.8 – 9.3 region. After evaporation, the amidation was conducted without purification in *N,N*-dimethylformamide (DMF) by addition of

Table 1. Automated synthesis of [^{18}F]FK960

Starting material	1a	1b
Fluorination	120°C, 10 min	150°C, 10 min
Preparation time	2 h 12 min–2 h 53 min	2 h 15 min–2 h 23 min
Radioactivity (MBq)	320–940	115–633
Radiochemical purity (%)	87–97	87–95
Specific activity (GBq/μmol)	2.0–3.1	13.0–60.2

the reagents to the mixture. However, the entire process from starting material took approximately 2 h owing to the two evaporation steps. Ethyl 4-trimethylammonium benzoate (**2a**) was first chosen as a starting material because of higher reactivity than the corresponding 4-nitro derivative.[10] The fluorination proceeded smoothly and the next two steps were also efficient. Consequently [^{18}F]FK960 was obtained in good yield. On the other hand, the material produced in this way had low specific activity. We considered the reason to be isotopic dilution from the starting material during the evaporation before neutralization. In order to avoid the use of the trifluoromethanesulfonate, ethyl 4-nitrobenzoate (**2b**) was chosen as the starting material. The fluorination reaction required 30°C higher temperatures than with compound (**2a**) and the amounts of side-product resulting from reaction of the product with dimethyl amine derived from DMF were increased. [^{18}F]FK960 with a specific radioactivity of 13.0–60.2 GBq/μmol was obtained (Table 1). The synthesis using ethyl 4-nitrobenzoate (**2b**) produced specific radioactivities 50–100 fold higher than when the trifluoromethanesulfonate (**2a**) was used.

PET study

The time activity curves shown in Figure 3 display the brain uptake of no-carrier added [^{18}F]FK960. After injection of [^{18}F]FK960, the radioactivity in whole blood and plasma decreased gradually, whereas the radioactivity in the brain increased gradually for 50 min and was maintained for 2 h. The radioactivity level in plasma was approximately 3 times higher than each ROI in the brain until 2 h after injection. No difference among the ROIs (the cerebellum, hippocampus, temporal cortex, occipital cortex, frontal cortex) was observed in the present study. FK960 is taken up gradually by the brain and is distributed to the whole brain. FK960 is a somatostatin releaser, however the present

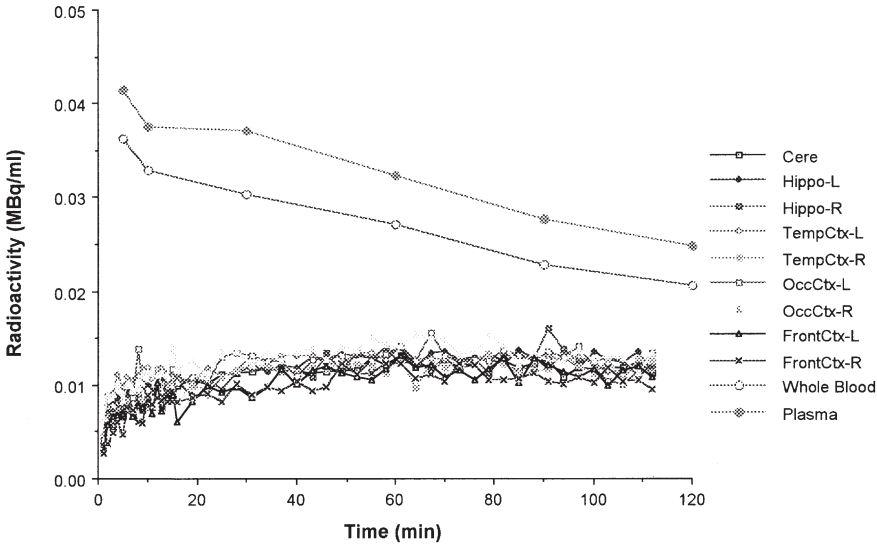

Figure 3. Time activity curves of [^{18}F]FK960 in brain and blood of conscious monkey

study did not show the specific binding site of FK960 in conscious monkey brain. Although the target molecule of FK960, and its distribution in the brain still remain to be elucidated, in the present study we could measure the distribution and pharmacokinetic data of FK960 in conscious monkey brain using PET. Furthermore, the present study suggests the possibility that using the same method, we will be able to obtain distribution and pharmacokinetic data in humans, which may provide us important information for clinical dose setting.

Experimental

Materials and reagents

Kryptofix222 was purchased from Aldrich Japan (Tokyo, Japan). Methyl sulfoxide (DMSO), NaOH 1N, and HCl 1N were purchased from Nacalai Tesque (Kyoto, Japan). Potassium fluoride, potassium carbonate, *N,N*-dimethylformamide (DMF), ammonium acetate, acetic acid and tetrahydrofuran (THF) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). 1-Hydroxybenzotriazole was purchased from Peptide Institute Inc (Osaka, Japan). WSC was purchased from Eiweiss (Shizuoka, Japan). DMSO was distilled under

vacuum from calcium hydride and kept over 4A molecular sieves. Preparative TLC was performed on Merck F-254 silica gel 60 plates (0.5 mm). Sterile filters were purchased from Millipore Japan (Tokyo, Japan). Compound (**2a**) was synthesized according to the literature.[10]

Analysis

1H-NMR spectra were recorded on an AC200P spectorometer (Bruker BioSpin K.K. Ibaraki, Japan). HPLC analysis was performed with a Tosoh CCPM-II pump (Tosoh Co., Ltd, Tokyo, Japan), an Aloka positron detector RLC-700 (Aloka Co., Ltd, Tokyo, Japan), a UV detector Tosoh UV-8020.

HPLC analysis of FK960

Column: Jasco finepak SIL C18S (4.6 mm × 150 mm, 5 μm) (Jasco Co., Ltd, Tokyo, Japan)

Mobile phase: (0.05 N NH_4OAc:AcOH = 250:1):THF = 87.5:12.5.

Flow rate: 1.0 ml/min

Synthesis of FK960

To a solution of potassium fluoride (3 mg, 52 μmol), potassium carbonate (3 mg, 22 μmol), and kryptofix222 (35 mg, 93 μmol), was added a solution of the starting material (ethyl 4-trimethylammonium-benzoate triflate (**2a**) (18.5 mg, 52 μmol) or ethyl 4-nitrobenzoate (**2b**)(9.2 mg, 52 μmol) in DMSO (0.6 ml). The mixture was heated at 120°C for 10 min (starting material **2a**) or 150°C for 10 min (starting material **2b**). The reaction mixture was cooled to room temperature. To this mixture was added 0.8 ml of 1N NaOH and stirred at 80°C for 5 min, and then the solvent was removed under reduced pressure. To this crude product was added 1-acetyl-6-aminopiperazine (**5**), (10.2 mg, 71 μmol in 0.8 ml 1 M HCl) and stirred at room temperature for 2 min. To the mixture was added 1-hydroxybenzotriazole (9.6 mg, 71 μmol) and WSC (13.6 mg, 71 μmol) in DMF (1.0 ml). The mixture was stirred at room temperature for 10 min, and then the solvent was removed in vacuo. The purification was performed on preparative TLC (10% MeOH–CH_2Cl_2 Rf: 0.3) to give FK960 (2.8 mg) (22.4%).

1H-NMR (DMSO-d6) d ppm 2.02(3H, s), 2.76–2.94(4H, m), 3.45–3.60(4H, m), 7.29(2H, t, J = 8.8 Hz), 7.84(2H, dd, J = 5.6, 8.8 Hz), 9.59(1H, s)

Synthesis of [^{18}F]FK960

Production of $^{18}F^-$ was accomplished via $^{18}O(p, n)^{18}F$ reaction by proton bombardment (18 MeV, 20 μA) of a ^{18}O–water target using a cyclotron-target system (CYPRIS HM-18,Sumitomo Heavy Industries Co., Ltd). After irradiation, the ^{18}F containing target water was transferred to a ^{18}F recovery system. The target material was passed through an AG1-X8 (Bio-Rad) anion exchange resin and recovered as [^{18}F]KF by elution with aqueous potassium carbonate (0.3 ml, 66 mM) followed by water (0.3 ml) into a conical glass vial. To this solution kryptofix2.2.2. (20 mg, 53 μmol) in acetonitrile was added. The recovered $^{18}F^-$ solution was then transferred to the synthesis apparatus using He gas. The radioisotope detector was used to monitor the arrival of radioactivity from the glass vial. The aqueous solution was removed azeotropically with acetonitrile at 120°C. This operation was repeated three times. To the residue was added a solution of the starting material (ethyl 4-trimethylammoniumbenzoate triflate (**2a**) (18.5 mg, 52 μmol) or ethyl 4-nitrobenzoate (**2b**)(9.2 mg, 52 μmol) in DMSO (0.6 ml). The mixture was heated at 120°C for 10 min (starting material **2a**) or 150°C for 10 min (starting material **2b**). After the reaction mixture was cooled to room temperature, 1N-NaOH (0.8 ml) was added. The mixture was heated 80°C for 5 min, and the solvent was evaporated by N_2 flush and reduced pressure. The residue was neutralized in a mixed solution of 1-acetyl-6-aminopiperazine (**5**), (10.2 mg, 71 μmol in 0.8 ml 1 M HCl) and water (0.5 ml). The mixture was stirred for 2 min at room temperature. To this mixed solution was added a mixed solution of 1-hydroxybenzotriazole (9.6 mg, 71 μmol) and 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (13.6 mg, 71 μmol) in DMF (1.2 ml). The mixture was stirred at room temperature for 10 min. After the reaction, the mixture was evaporated by N_2 flush and reduced pressure in a similar manner. A mixture of water and methanol was added to the reaction vessel and the residue was dissolved and the solution was loaded on to an HPLC column. The fraction containing [^{18}F]FK960 was collected in the evaporator. The eluate was evaporated by N_2 flush and reduced pressure. The residue was dissolved with saline

and passed through a sterile filter into a sterile vial. The retention time of the product (6.8 min) was identical to that of the authentic sample.

Preparative HPLC was performed with a Rabo-Quortec pump system (Rabo- Quortec Co., Ltd Tokyo, Japan).

Column: Jasco Megapak SIL C18-10 (7.5 mm × 250 mm, 5 μm)

Mobile phase: (0.05N NH_4OAc:AcOH = 250:1):THF = 87.5:12.5.

Flow rate: 4.0 ml/min

Retention time: FK960 (8.5 min)

PET study

Three young-adult male rhesus monkeys (*Macaca mulatta*) weighing from 4 to 6 kg were used for the PET measurements. Monkeys were maintained and handled in accordance with recommendations of the US National Institutes of Health and also the guidelines of the Central Research Laboratory, Hamamatsu Photonics. They were trained to sit on a chair twice a week over a period of more than 3 months. Magnetic resonance images (MRI) of all monkeys were obtained with a TOSHIBA MRT-50A/II (0.5 T) under pentobarbital anesthesia. The stereotaxic coordinates of PET and MRI were adjusted based on the orbitomeatal (OM) line with a specially designed head holder.[11] At least 1 month before the PET study, an acrylic plate, with which the monkey was fixed to a monkey chair, was attached to the head under pentobarbital anesthesia as described previously.[12]

Data were collected on a high-resolution PET scanner (SHR-7700, Hamamatsu Photonics, Hamamatsu, Japan) with transaxial resolution of 2.6 mm full width at half maximum (FWHM) and a center-to-center distance of 3.6 mm.[13] The PET camera allowed 31 slices for imaging to be recorded simultaneously.

After an overnight fast, animals were fixed to the monkey chair with stereotaxic coordinates aligned parallel to the OM line. A cannula was implanted into the posterior tibial vein of the monkey for administration of [^{18}F]FK960, and another cannula was put into the femoral vein of the other leg to obtain blood samples. PET scans were performed under dim light.

[^{18}F]FK960 (100–120 MBq/kg body weight) was injected through the posterior tibial vein cannula, and PET scan was performed for 112 min with 16 time frames at 1 min intervals, followed by 32 time frames at 3 min.

Regions of interest (ROIs), i.e. the cerebellum, hippocampus, temporal cortex, occipital cortex, frontal cortex, were identified according to MR images of each monkey brain, and time activity curves in ROIs were obtained.

Blood samples were obtained 5, 10, 30, 60, 90 and 120 min after [^{18}F]FK960 injection. Blood samples were centrifuged to separate plasma, weighed, and the radioactivity was measured.

Conclusion

In the present study, we developed a one-pot reaction for the synthesis of [^{18}F]FK960, and succeeded to prepare this labelled drug as an injectable solution, and obtained the distribution and pharmacokinetic data in conscious monkey brain using a [^{18}F]FK960 PET study. After injection of [^{18}F]FK960, the radioactivity in the brain increased gradually for 50 min and then was maintained up to 2 h. The radioactivity level in plasma was 3 times as high as each ROI in the brain. Differences between ROI were not observed in this study. The present study suggests the possibility to measure distribution and pharmacokinetic data in humans using the same method.

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References

1. Yamazaki M, Matsuoka N, Maeda N, Ohkubo Y, Yamaguchi I. *J Pharmacol Exp Ther*. 1996; **279**: 1157–1173.
2. Matsuoka N, Aigner TG. *J Pharmacol Exp Ther* 1997; **280**: 1201–1209.
3. Matsuoka N, Satoh M. *Brain Res* 1998; **794**: 248–254.
4. Inoue T, Wang F, Moriguchi A, Shirakawa K, Matsuoka N, Goto T. *Brain Res* 2001; **892**: 111–117.
5. Araujo DM, Lapchak PA, Collier B, Quirion R. *J Neurochem* 1990; **55**: 1546–1555.
6. Tsukada H, Yamazaki S, Noda A. *et al*. *Brain Res* 1999; **832**: 118–123.

7. Angelini G, Speranza M, Wolf AP, Shiue C.-Y. *J Fluorine Chem* 1985; **27**: 177–192.
8. Attina M, Cacace F, Wolf AP. *J Chem Soc Chem Commun* 1983; 108–109.
9. Shiue C-Y, Watanabe M, Wolf AP, Fowler J, Salvadori P. *J Label Compd Radiopharm* 1984; **21**: 533–547.
10. Haka, MS., Kilbourn, MR., Watkins, GL, Toorongian, SA. *J Label Compd Radiopharm* 1988; **27**: 823–833.
11. Takechi H, Onoe H, Imamura K. *Neurosci Lett* 1994; **182**: 279–282.
12. Onoe H, Inoue O, Suzuki K, *et al. Brain Res* 1994; **663**: 191–198.
13. Watanabe M, Okada H, Shimizu K, *et al. IEEE Trans Nucl Sci* 1997; **44**: 1277–1282.

