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Synthesis of [^{18}F]fallypride in a micro-reactor:

rapid optimization and multiple-production in small doses for micro-PET studies

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Abstract

A commercial coiled-tube micro-reactor (NanoTek; Advion) was used as a convenient platform for the synthesis of [^{18}F]fallypride in small doses (0.5–1.5 mCi) for micro-PET studies of brain dopamine subtype-2 receptors in rodents. Each radiosynthesis used low amounts (20–40 μg ; 39–77 nmol) of tosylate precursor and [^{18}F]fluoride ion (0.5–2.5 mCi). Optimization of the labeling reaction in the apparatus, with respect to the effects of precursor amount, reaction temperature, flow rate and [^{18}F]fluoride ion to precursor ratio, was achieved rapidly and the decay-corrected radiochemical yield of [^{18}F]fallypride (up to 88%) was reproducible. The low amounts of material used in each radiosynthesis allowed crude [^{18}F]fallypride to be purified rapidly on an analytical-size reverse phase HPLC column, preceding formulation for intravenous injection. Scale-up of the reaction was easily achieved by continuously infusing reagent precursor solutions to obtain [^{18}F]fallypride in much greater quantity.

Keywords

Micro-reactor; fluorine-18; radiofluorination; [^{18}F]fallypride; synthesis

Introduction

Recently, micro-reactor (or microfluidic) devices have emerged as being extremely useful for the intensification and miniaturization of chemical processes.^{1–4} These devices often consist of a network of micron-sized channels (typical cross-sections in the range 10–300 μm), incorporating a selection of reaction loops/chambers, filters, separation columns and electrodes etched onto a solid substrate (e.g., glass, plastic). Alternatively, micro-reactors have been constructed from narrow-bore tubing.^{5–7} The ability to manipulate and analyze reactions in both space and time within the channel network or tubing of a micro-reactor provides for a fine level of reaction control. It has become increasingly recognized that micro-reactors may present considerable advantages in radiochemistry with short-lived positron-emitters (e.g., carbon-11, $t_{1/2} = 20.4$ min; fluorine-18, $t_{1/2} = 109.7$ min) to produce radiotracers or radioligands reliably and reproducibly for molecular imaging in living animal and human subjects with positron emission tomography (PET).^{8–17} Several advantages may be expected from this technology, including (1) the use of lower amounts of materials (especially non-radioactive precursor, which may be precious or difficult to obtain), (2) easier and more efficient product purification,

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(3) greater conservation of radioactive product and its specific radioactivity, and (4) reduced radiation exposure to radiochemists by working with less radioactivity.

Various examples of microfluidic PET radiochemistry have focused on reactor design and proof-of-principle reactions, as in the reported syntheses of ^{11}C - and ^{18}F -labeled carboxylic esters¹¹ and ^{11}C -labeled amines^{12,13}. In further examples, full radiosyntheses of the important PET radiotracer, [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG), have been accomplished.^{14–17} Most of these studies have reported the radiochemical yields under various conditions, but none has investigated the specific radioactivities of the labeled products.

[^{18}F]Fallypride is widely used for PET imaging of dopamine subtype-2 (D_2) receptors in living animal^{18,19} or human brain.²⁰ The one-step radiosynthesis of [^{18}F]fallypride from the reaction of [^{18}F]fluoride ion with a tosylate precursor²¹ (Scheme 1) in conventional PET radiochemistry apparatus requires lengthy purification with HPLC because of significant thermal degradation of the precursor and formation of side products, which is used in ~ 2 mg (4- μmol) quantities. Here we demonstrate the utility of a commercial micro-reactor device (NanoTek; Advion) (Scheme 2) for the optimization of [^{18}F]fallypride synthesis and the production of this radioligand in small doses (0.5–1.5 mCi) at high specific radioactivity and purity for rodent imaging and metabolic studies.

Results and Discussion

Initially, a series of reactions was performed in the micro-reactor apparatus in order to establish conditions in which [^{18}F]fallypride could be produced rapidly and in high radiochemical yields. The flow rates of the two reagents, precursor solution and [^{18}F]fluoride ion solution, were generally set to be equal and determined the reaction time, which equates to the residence time of the reagents within the heated reactor coil(s) (Scheme 2). Reactions were quenched by dilution of the reactor output with a water-acetonitrile (1: 1 v/v) mixture at room temperature before analysis by radio-HPLC or radio-TLC. Under normal conditions the efficiency of radioactivity recovery from the reactor was about 85%.

In a 2-m length reactor, the decay-corrected radiochemical yield (RCY) of [^{18}F]fallypride increased from 0 to 65% over the temperature range 100–170 °C (Fig. 1).^{*} RCYs of [^{18}F]fallypride were quite reproducible and independent of laboratory setting and method of analysis (radio-HPLC or radio-TLC). Each radiosynthesis used 20 or 40 μg (38.7 or 77.4 nmol) of precursor, which is only 1 or 2% of that used conventionally by others,²¹ and also by us²². A whole run required only 4 min. This included the simultaneous delivery of 10 μL of solution at 10 $\mu\text{L}/\text{min}$ from each reactant storage loop to the reactor, followed by a sweep out of the reaction mixture at 30 $\mu\text{L}/\text{min}$ to the collection vial with a chase bolus of acetonitrile (86 μL). The sweep volume was set to exceed the internal volume of the tubing connecting the end of the reactor to the distribution valve, and then to the final collection vial. The reaction/residence time for a particular liquid plug inside the 2-m reactor was only 94 s at 10 $\mu\text{L}/\text{min}$ infusion rate. Hence, it was possible to perform several reactions in quick succession under controlled and varied conditions. Reduced and erratic RCYs occurred at temperatures over 170 °C and were most likely due to the greater difficulty of maintaining liquid phase (acetonitrile, b.p. = 89 °C) in the reactor and smooth solvent flow.

RCYs were also influenced by the total length of reactor tubing, the flow rate and the reactant volume ratio. By the use of two 2-m length reactors (total length = 4 m) connected in series, RCYs reached 88% (Table 1). In a single 4-m reactor, a longer residence time, achieved by

^{*}The reaction temperatures cited here are the set heater temperatures; a temperature gradient may exist between the heater and the inside of the micro-reactor, and hence true reaction temperatures may be somewhat lower than cited.

using a slower flow rate from each reactant syringe, also increased the RCY (Fig. 2). When the volume ratio of [^{18}F]fluoride ion to precursor solution was increased RCY increased slightly and when this ratio was decreased RCY decreased slightly (Fig. 2).

The very low amounts of precursor, base (K_2CO_3) and kryptand (K 2.2.2) routinely used in the micro-reactor greatly restricted the possibilities for forming appreciable quantities of unknown chemical impurities. Thus, HPLC analysis of crude radioactive products with eluate monitored for absorbance at 305 nm, revealed only low level chemical impurities (Fig. 3, **upper panel**). The crude radioligand was readily and rapidly purified on an analytical-size reverse phase HPLC column to provide radiochemically and chemically pure [^{18}F]fallypride for formulation and subsequent intravenous injection into small animals (Fig. 3, **lower panel**). For mCi-level production of radioligand, the HPLC purification was made easier after removal of inorganic material by 10-fold dilution of the reactor output with water and passage through a SPEC C18 AR pipette tip.

Higher activities of [^{18}F]fallypride, as needed for PET studies for human injection (5–20 mCi) were readily accessible through the scale-up of this process. Thus, a large amount of [^{18}F]fallypride (> 13 mCi) was obtained in a single batch by continuously infusing greater volumes of [^{18}F]fluoride ion and precursor solution (200 μL each) through the reactor under optimal conditions (170 $^{\circ}\text{C}$ and 10 $\mu\text{L}/\text{min}$). In this case, the radioligand was produced in 0.4 mL of acetonitrile after 23 min with a RCY of 88% by radio-TLC (Table 1). Multi-Ci levels of [^{18}F]fluoride ion can now be produced on modern compact cyclotrons by the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ reaction on [^{18}O]water.^{23,24} Hence, in principle, multiple high doses of [^{18}F]fallypride might be produced in the same micro-reactor apparatus.

For neuroimaging with [^{18}F]fallypride in small animals, the amount of non-radioactive fallypride co-injected with radioligand needs to be below that which will cause an appreciable occupancy of brain D_2 receptors;²⁵ therefore, the specific radioactivity of the [^{18}F]fallypride needs to be high. The method by which cyclotron-produced [^{18}F]fluoride ion was recovered from irradiated [^{18}O]water and dried had significant impact on the final specific radioactivity of the radioligand. Initially, [^{18}F]fluoride ion was recovered and dried with the drying station of the Nanotek apparatus (the CE module). This module used a cartridge of either an MP-1 or QMA anion exchange resin to adsorb the [^{18}F]fluoride ion for later elution in acetonitrile-water containing potassium carbonate and K 2.2.2. The [^{18}F]fallypride produced from [^{18}F]fluoride ion that had been dried in this manner was found to have a rather low specific radioactivity (Table 2). When the cyclotron-produced [^{18}F]fluoride ion was dried without the use of a cartridge, but through cycles of evaporation with added acetonitrile, the specific radioactivity of the prepared [^{18}F]fallypride was substantially higher, and was similar to that which we achieved in an alternative radiosynthesis in a more conventional apparatus (Synthia).²⁶ Use of an MP-1 or QMA anion exchange resin to dry [^{18}F]fluoride ion preceding the conventional radiosynthesis in Synthia also dramatically reduced the specific radioactivity of the product. This confirmed that there was low-level contamination of the MP-1 and QMA anionic resins with fluoride ion. It is expected that this problem will be solved by improvements to the process for preparing the resin cartridges.

Experimental

Materials

'Tosyl-fallypride' ((*S*)-*N*-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-toluenesulfonyloxypropyl)-2,3-dimethoxybenzamide) and fallypride ((*S*)-*N*-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-fluoropropyl)-2,3-dimethoxybenzamide) were purchased from ABX (Radeberg; Germany). Kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8.8.8]hexacosane, K 2.2.2; 98%), ammonium formate (99.995%), potassium carbonate (99%)

and acetonitrile (anhydrous, 99.8%) were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received. Acetonitrile (high purity solvent, Burdick & Jackson; Morristown, NJ) for HPLC mobile phase was also used without further treatment. NCA [^{18}F]fluoride ion was obtained through the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction by irradiating [^{18}O]water (95 atom %) for 120 min with a proton beam (14.1 MeV; 20 μA) produced by a PETrace cyclotron (GE, Milwaukee, WI). MP-1 or QMA anionic resin cartridges were supplied by ORTG (Oakdale, TN).

General methods

Radiosyntheses were performed in lead-shielded hot-cells. Radioactivity was measured with a calibrated dose calibrator (Atomlab 300; Biodex Medical Systems Inc., Shirley, NY). HPLC analyses of crude reaction mixtures were performed on a system comprising a solvent module (System Gold 126; Beckman Coulter, CA) coupled with a UV absorbance detector (Model 168; Beckman Coulter) and a radioactivity detector (PMT, Flow-count; Bioscan, Washington, DC). Analysis of [^{18}F]fallypride prepared for injection into rodents was carried out with the same HPLC system. Column and elution conditions are described later. The response of the absorbance detector at 305 nm was calibrated with reference fallypride so that the mass of carrier in the injectate could be calculated. The amount of radioactivity associated with the carrier peak was measured to permit the specific radioactivity of the radioligand to be derived. LC-MS for compound characterization was performed on a LCQ Deca instrument (Thermo Fisher Scientific Corp., Waltham, MA) equipped with a Synergi Fusion-RP column (4 μm , 150 $\times$ 2 mm i.d.; Phenomenex, Torrance, CA).

Preparation of [^{18}F]fluoride ion reagent

Cyclotron-produced no-carrier-added [^{18}F]fluoride ion (10–200 mCi) in [^{18}O]water (50–400 μL) was first adsorbed onto a small anion resin (i.e., MP-1 or QMA) cartridge within the CE module of a NanoTek apparatus (Advion; Louisville, TN), and then released with a solution of K_2CO_3 (5.5 mg/mL) plus K 2.2.2 (30 mg/mL) in $\text{MeCN-H}_2\text{O}$ (9: 1 v/v; 150 μL) into a 5-mL V-vial. The solution was dried by two cycles of azeotropic evaporation with acetonitrile (0.5 mL) at 100 $^\circ\text{C}$. Alternatively, for drying with microwaves, no-carrier-added [^{18}F]fluoride ion (10–200 mCi) in [^{18}O]water plus a solution of K_2CO_3 (5.5 mg/mL) and K 2.2.2 (30 mg/mL) in $\text{MeCN-H}_2\text{O}$ (9: 1 v/v; 150 μL) were placed in a 5-mL V-vial. The solution was dried by four cycles of azeotropic evaporation with acetonitrile (0.65 mL per addition) at 90 W for 2 min in a microwave cavity (521-type; Resonance Instrument Inc., Skokie, IL) housed within a modified Synthia module.²⁶ Dry [^{18}F]F $^-$ -K $^+$ -K 2.2.2 reagent was dissolved in acetonitrile for radiosynthesis.

Configuration and operation of the NanoTek apparatus

The NanoTek apparatus consists of two modules: the concentrator (CE) and the micro-reactor base (LF) modules. The set-up of the LF module used in this work is depicted in Scheme 2. A single micro-reactor is composed of coiled silica glass tubing (e.g., length, 2 m; internal diameter, 100 μm ; internal volume, 15.7 μL), housed in a brass ring filled with a poly(silicone) that is resistant to high-temperature. A reactor may be used alone or in series with another. Each reactor may be heated and thermostatted to a set temperature. The reactors may be fed with reagents from storage loops at set flow rates, by the action of mechanically-driven syringes. A reactor may operate up to a pressure of 300 psi, so allowing a solvent to be used well above its boiling point. Reactor pressure is monitored, and if it exceeds 750 psi, due to a blockage or to an excessive flow rate, power is shut down to protect the instrument.

The apparatus was cleaned by flushing tubes and reactors with acetonitrile at the beginning of each experimental session and again at the end. Reagent solutions were each loaded into their respective storage loops from different source vials. To perform one reaction, 10–20 μL of

solution from each loop were infused (via valve connection from respective syringe pump → E → D → C) into the micro-reactor at a set flow rate and temperature. A bolus of acetonitrile was then infused from syringe pump 2 (via valve connection from syringe pump 2 → B) to sweep all reaction mixture out of the reactor and into the collection vial via the distribution valve. The volume of the sweep solvent was set to just exceed the aggregate internal volume of the reactor and subsequent transfer tubing. Once the source vials were in place, all operations were programmed to run in sequence automatically and remotely.

Optimization of [^{18}F]fallypride synthesis in micro-reactor

Dry [^{18}F]F $^{-}$ -K $^{+}$ -K 2.2.2 solution (10–200 mCi) and tosylate precursor (0.51 mg; 0.99 μmol), both in acetonitrile (255 μL), were loaded into their respective storage loops in the micro-reactor apparatus. Then 10–20 μL of solution from each loop were infused simultaneously at set flow rates into the thermostatted reactor. Reactions were quenched by immediately adding the reactor effluent to MeCN-H $_2$ O (1: 1 v/v; 1 mL) at room temperature. RCYs were calculated from TLC or HPLC analysis. Radio-TLC was performed on silica layers (Alltech, IL) developed with MeCN-H $_2$ O (9: 1, v/v), and radioactive compounds quantified with a TLC radio-imaging scanner (AR200; Bioscan). Radio-HPLC was performed on a reverse phase column (Prodigy ODS 3, 5 μm , 100 $\text{\AA}$, 250 $\times$ 4.6 mm i.d.; Phenomenex) eluted at 0.85 mL/min with 25 mM HCOONH $_4$ in MeCN-water (1: 1 v/v) ([^{18}F]fallypride, t_{R} = 6.4 min).

Production of [^{18}F]fallypride in micro-reactor for micro-PET studies

Dry [^{18}F]F $^{-}$ -K $^{+}$ -K 2.2.2 (10–200 mCi) and tosylate precursor (0.51 mg; 0.99 μmol), each dissolved in acetonitrile (255 μL), were loaded into their respective storage loops in the micro-reactor apparatus. Solution from each loop (10 μL) was infused simultaneously at 10 $\mu\text{L}/\text{min}$ into the reactor (length, 4 m) held at 140 $^{\circ}\text{C}$. The reaction mixture plus sweep solvent (acetonitrile $\sim$ 100 μL) was injected onto a reverse phase column (Luna C18, 5 μm , 100 $\text{\AA}$, 250 $\times$ 4.6 mm i.d.; Phenomenex) eluted at 1 mL/min with MeCN-*aq.* 25 mM HCOONH $_4$. The gradient started at 45% B for 7 min, gradually increased to 70% B over 8 min and was then kept at 70% B for 5 min. The fraction eluting between 10.9–11.9 min was collected ([^{18}F]fallypride, t_{R} = 11.3 min). Mobile phase was removed by dilution of the collected fraction with water (10 mL) and passage through a SPEC C18 AR SPE (Varian, Lake Forest, CA) pipette tip. [^{18}F]Fallypride was eluted off the SPE pipette tip with ethanol (100 μL) and formulated in saline for injection (1.0 mL).

The formulated [^{18}F]fallypride was analyzed on the same type of column as used in separation, but using MeCN-*aq.* 25 mM HCOONH $_4$ (65: 45 v/v) as mobile phase at 1 mL/min ([^{18}F]fallypride, t_{R} = 5.5 min). Radioligand identity was confirmed 1) by co-injection of a sample of the formulated [^{18}F]fallypride with carrier fallypride onto the radio-HPLC system and verification of co-elution, and 2) by LC-MS of associated carrier [m/z = 365.2 ($\text{M}+1$) $^{+}$] in the formulated preparation.

The overall radiosynthesis time, including [^{18}F]fluoride ion drying (20 min), pre-heating the reactor (5 min), reaction (4 min), product purification on HPLC (15 min) and formulation (5 min), was about 50 min.

Synthesis of [^{18}F]fallypride in Synthia apparatus

Synthia is a programmable semi-robotic radiosynthesis device.²⁷ Batches of [^{18}F]fallypride were produced in a Synthia apparatus from two sources of [^{18}F]fluoride ion reagent, generated either by recovery on ion exchange resin or by microwave drying, as detailed above. The radiosynthesis used 1.0 mg (2 μmol) of tosylate precursor in acetonitrile (0.5 mL) with microwave heating (55 W, 2 $\times$ 2 min) of the labeling reaction. [^{18}F]Fallypride (t_{R} = 17 min) was obtained in adequate chemical purity for use in micro-PET studies by purification of the

crude product on a 'semi-preparative' size reverse phase column (Luna C18, 5 μ m, 250 $\times$ 10 mm i.d.; Phenomenex) eluted with MeCN-*aq.* 25 mM HCOONH₄ (43: 57, v/v) at 3 mL/min. Other aspects of the radiosynthesis were the same as that described in detail within the CMC (Chemistry, Manufacturing and Controls) section of our 'exploratory Investigational New Drug' submission to the United States Food and Drug Administration, which is accessible on the web.²²

Conclusions

A commercial microfluidic radiosynthesis apparatus enabled rapid optimization of the labeling of fallypride with fluorine-18 and was successfully exploited to produce adequate activities of [¹⁸F]fallypride for micro-PET applications. The mode of preparing the [¹⁸F]fluoride ion reagent was critical for achieving high specific radioactivity. The use of low amount of materials, especially of the tosylate precursor, greatly eased the challenge of radioligand purification. The process was scaleable to produce higher activities of [¹⁸F]fallypride, as desired.

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Scheme 1.
Synthesis of $[^{18}\text{F}]$ fallypride in a single-step from $[^{18}\text{F}]$ fluoride ion.²¹

Scheme 2.

Set-up of micro-reactor, reagent storage and delivery, product collection valves, and flow directions for $[^{18}\text{F}]$ fallypride synthesis in the NanoTek apparatus.

Fig. 1.
Effect of reaction temperature on RCY of [^{18}F]fallypride synthesized in a 2-m micro-reactor. Reaction on a 10- μL scale was carried out in Knoxville, TN; reaction on 20- μL scale was carried out at NIMH.

Fig. 2.

Influence of $[^{18}\text{F}]$ fluoride ion/precursor solution ratio (v/v) and flow rate on RCYs of $[^{18}\text{F}]$ fallypride at 140 °C in a 4-m micro-reactor. In these experiments, the dispensed volume of $[^{18}\text{F}]\text{F}^-\text{K}^+\text{K} 2.2.2$ solution was fixed at 10 μL and its flow rate set at 5, 10 or 20 $\mu\text{L}/\text{min}$. The volume ratio of precursor solution to $[^{18}\text{F}]\text{F}^-\text{K}^+\text{K} 2.2.2$ solution was adjusted by varying the precursor solution flow rate, accordingly. Thus, for example, when the ratio was 2, 20 μL of precursor solution and 10 μL of $[^{18}\text{F}]$ fluoride ion solution were infused simultaneously.

Fig. 3. Preparation of [^{18}F]fallypride for intravenous injection: separation and analysis. Upper panel: chromatogram of the reaction mixture obtained by infusion of $^{18}\text{F}/\text{K}^+-\text{K}$ 2.2.2 solution and precursor solution (10 μL each) into the micro-reactor at 140 $^{\circ}\text{C}$. The product fraction eluting between 10.9–11.9 min was collected for formulation. Lower panel: chromatogram from the analysis of formulated [^{18}F]fallypride. Both chromatographies used a reverse phase column (Luna C18, 5 μm , 250 $\times$ 4.6 mm i.d.; Phenomenex), but with different elution conditions (see Experimental section).

Example of sequential radiosyntheses of [¹⁸F]fallypride in two coupled 2-m micro-reactors, starting with [¹⁸F]fluoride ion solution (0.34 mCi/μL) in the apparatus at 15:00 h.

Table 1

Synthesis number	Reactor temperature (°C)	Infused volume of [¹⁸ F]fluoride ion solution (μL)	Time of reaction completion	Activity of reaction mixture (mCi)	RCY of [¹⁸ F]fallypride (%)
1	190	10	17:00 h	1.43	16
2	170	10	17:04 h	1.40	81
3	150	10	17:08 h	1.38	73
4	170	200	18:38 h	15.4	88

Table 2

Specific activity of [^{18}F]fallypride produced through different drying protocols (thermal or microwave) for [^{18}F]fluoride ion reactant and with different apparatus (Nanotek LF or Synthia).

Fluoride drying protocol	Labeling method	Specific radioactivity (mCi/ μmol)*
MP1 in CE	Thermal in LF	860 ± 292 ($n = 4$)
MP1 in CE	MW in Synthia	555 ± 245 ($n = 3$)
QMA in CE	MW in Synthia	704
No cartridge in CE	MW in Synthia	5,101
MW in Synthia	Thermal in LF	$4,248 \pm 1,190$ ($n = 4$)

Grouped data are mean $\pm$ SD.

MP1 = MP1 anion exchange resin cartridge; QMA = QMA anion exchange resin cartridge; CE = NanoTek concentrator module; LF = NanoTek micro-reactor base module; MW = Microwave.

* For ease of comparison, all specific radioactivities are decay-corrected to the end of radionuclide production.