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Initial experience in synthesis of (2*S*,4*R*)-4-[^{18}F]fluoroglutamine for clinical application

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Abstract

We report initial experience in synthesis of (2*S*,4*R*)-4-[^{18}F]fluoroglutamine, [^{18}F]FGln, which has been used as a tool for monitoring glutamine metabolism in cancer patients. [^{18}F]FGln was prepared by a fully-automated PET-MF-2V-IT-I synthesizer under GMP-compliant conditions for routine clinical studies. The total radiosynthesis time was about 65 min, the decay-corrected radiochemical yield was 18.0 ± 4.2% (n = 59; failure n = 15) and the radiochemical purity was greater than 90%. In some situations, the yields were low (< 5%) and the most likely cause of this problem is the initial fluorination step, the fluoride ion might not have been fully activated. In other occasions, low final radiochemical purity was often associated with the failure of second step - removal of protection groups by anhydrous trifluoroacetic acid. A trace amount of water led to production of undesired 4-[^{18}F]fluoroglutamic acid. Knowledge learned from the successes and failures of synthesis may be helpful to identify critical steps and pitfalls for preparation of this clinically useful metabolic probe, [^{18}F]FGln, for imaging glutamine utilization in tumor of cancer patients.

Keywords: Cancer metabolism, PET imaging, tumor, glutamine and radiolabelling

1. Introduction

Position emission tomography (PET) is a widely used clinical imaging device for cancer diagnosis and monitoring. In conjunction with 2-[^{18}F]fluoro-2-deoxy-glucose ([^{18}F]FDG), it is a common imaging procedure for mapping cellular glucose metabolism in different tissues and organs. The uptake of [^{18}F]FDG, especially in tumors with higher rates of glycolysis, provides an accurate assessment of cellular glucose metabolism with high signal to noise ratios. Despite the tremendous promise of using [^{18}F]FDG-PET to detect and monitor tumor metabolism, in many cases tumors are [^{18}F]FDG-negative; the tumors may have cleverly changed their nutrient sources from glucose to glutamine, thus escaping detection by PET imaging.[1] To improve cancer diagnosis based on energy metabolism we have prepared and tested a series of novel fluorinated glutamine derivatives mimicking glutamine, which is fueling the tumor growth.[2,3] Four different isomers (2*S*,4*S*; 2*S*,4*R*; 2*R*,4*S*; 2*R*,4*R*) of 4-[^{18}F]fluoroglutamine were tested by *in vitro* cell uptake assays and *in vivo* PET imaging in rats.[4] Only the 2*S* isomers, the naturally occurring glutamine configuration, showed a significant glutamine-specific uptake. Using product with correct optical purity is essential for the success of glutamine imaging. Cell uptake in SF188 and 9L glioblastoma tumor cells displayed a higher rate of uptake, which was higher than that of [^{18}F]FDG.[5] Dynamic small animal PET imaging studies of (2*S*,4*R*)-4-[^{18}F]fluoroglutamine ([^{18}F]FGln) were conducted in 9L tumor-cell-xenografts produced in F344 rats. Biodistribution and PET imaging studies showed that [^{18}F]FGln localized in tumors with a higher uptake than in surrounding muscle and liver tissues.[5] In addition, at 30 and 60 min after an iv injection into tumor bearing rats, [^{18}F]FGln displayed a high incorporation (30% and 70%, respectively) into macromolecules suggesting a rapid intracellular metabolism.

Recent reports suggested that [^{18}F]FGln may be useful for detecting and monitoring tumor metabolism based on glutamine utilization.[2,6-10] After an iv injection [^{18}F]FGln demonstrated good uptake and a high tumor-background ratio in patients. Results of human PET imaging with [^{18}F]FGln suggested that it is an attractive alternative tracer, other than commonly used [^{18}F]FDG, for clinical diagnosis and therapy evaluation. [2,6-12]

In order to perform further studies using [^{18}F]FGln, we report herein the experience, successes as well as failures, in the past one year and five months. This was about using

PET-MF-2V-IT-I modular synthesizer for routine preparation of [^{18}F]FGln in the PET/Cyclotron facility of Peking University Cancer Hospital for clinical application.

2. Materials and methods

The tosylate precursor of [^{18}F]FGln, (2*S*,4*S*)-tert-butyl-2-(tert-butoxycarbonylamino)-5-oxo-4-(tosyloxy)-5-(2,4,6-trimethoxybenzyl-amino)pentano-ate ($[\alpha]^{25}_{D}$ = −56.01 (c = 0.1, Methanol)) and non-radiolabeled reference standard, (2*S*,4*R*)-4-fluoroglutamine ([^{19}F]FGln), were prepared as described previously.[4] All other reagents and solvents were commercial products and were used without further purification unless otherwise indicated. Solid-phase extraction cartridges (Oasis HLB 3cc cartridge, QMA light cartridge) were obtained from Waters (Milford, MA, USA). AG 11 A8 Resin was obtained from Bio-Rad (Hercules, CA, USA). Sterile Millex-GS filter (SLGSV255F) was obtained from EMD Millipore (Billerica, MA, USA). The synthesis of [^{18}F]FGln was performed by a PET-MF-2V-IT-I synthesizer (PET Co. Ltd., Beijing, China) with two reaction vessels (Fig. 1). High-performance liquid chromatography was performed on an Agilent 1260 Infinity system with a chiral column (Chirex 3126 (D)-penicillamine, 250 × 4.6mm, Phenomenex). Gas chromatography was performed on an Agilent 6890 system with a FID detector. Radioactivity was measured by a CRC-25PET radioisotope dose calibrator (Capintec, Inc., Ramsey, NJ, USA). The clinical research study was approved by the Peking University Cancer Hospital &Institute Ethics Committee (Permission No. 2017KT38).

2.1 Preparation before radiosynthesis

To initiate the synthesis of [^{18}F]FGln on the synthesizer (Fig.1), reagent vials were added with desired reagents: 1.44 mg $KHCO_3$ and 8 mg 18-crown-6 in 1 mL methanol and 0.18 mL water were added to Vial 1;[13] 2 mL anhydrous acetonitrile was added to Vial 2; 5 mg precursor in 0.7 mL anhydrous acetonitrile was added to Vial 3; 30 mL sterile water was added to Vial 4; 10 mL sterile water/ethanol (8/2, v/v) was added to Vial 5; 1.5 mL anhydrous acetonitrile was added to Vial 6; 1 mL trifluoroacetic acid (TFA) and 10 μL

anisole were added to Vial 11; 2 mL sterile water for injection was added to Vial 12; 3 mL sterile water for injection was added to Vial 13.

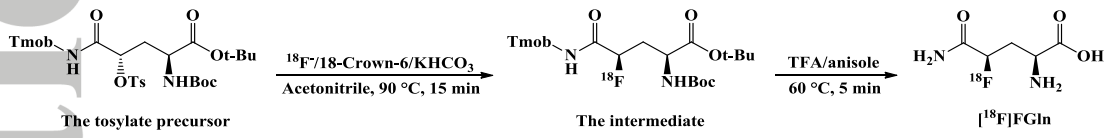
Preconditioning is necessary for several cartridges before radiosynthesis. QMA light cartridge was treated with 10 mL 0.5 M $NaHCO_3$ solution and 10 mL water. Oasis HLB 3cc cartridge was preconditioned with 10 mL ethanol and followed by rinse with 10 mL water. Weighted 2 g AG 11 A8 resin, was loaded before the HLB 3cc cartridge, after which the top was closed with a sleeve stopper. HLB-AG 11 A8 column was washed with 10 mL water. All conditioned cartridges were kept wet unless otherwise specified. Load QMA light cartridge to a specific location. Load Oasis HLB 3 cc cartridge between Valve 7 and Valve 10. Load HLB-AG 11 A8 column between Valve 15 and Valve 17. Valve 17 was connected through a sterile Millex-GS filter to a 10 mL sterile vial (final product vial).

2.2 Automated radiosynthesis of [^{18}F]FGln

[^{18}F]FGln was prepared using the two-step radiochemical reaction described in Scheme 1. [^{18}F]fluoride (37–148 GBq, 1–4 Ci), produced by a cyclotron using the $^{18}O(p,n)^{18}F$ reaction, was trapped on a Sep-Pak Light QMA cartridge. The ^{18}F-activity was eluted with phase transfer catalyst solution (8 mg 18-crown-6 and 1.44 mg $KHCO_3$ in 1 mL methanol and 0.18 mL water, Vial 1) from the cartridge into reactor 1 (H1), and evaporated under a stream of N_2 at 90 °C. 1 mL anhydrous acetonitrile in Vial 2 was added and the activity was azeotropically dried at 90 °C under a stream of N_2. 1 mL anhydrous acetonitrile in Vial 2 was added again and the activity was further dried at 90 °C under a stream of N_2. R1 was cooled to 50 °C, the tosylate precursor in Vial 3 was added to the dried activity. The resulting solution was stirred and heated at 90 °C for 15 min. R1 was cooled to 45 °C, the reaction mixture was diluted with 10 mL water (Vial 4), and passed through an Oasis HLB 3cc cartridge. The cartridge was washed twice with water (2 × 10 mL, Vial 4) and water/ethanol (8/2, v/v, 10 mL, Vial 5) . The intermediate was eluted from the cartridge with 1.5 mL acetonitrile (Vial 6) into reactor 2 (H2).

The eluted intermediate was heated at 60 °C under a stream of N_2 to remove acetonitrile. TFA/anisole mixture (1 mL/10 μL, Vial 11) was added to the dried residue. The resulting solution was heated at 60 °C for 5 min. The volatiles were removed at room

temperature under a stream of N_2. 2 mL sterile water (Vial 12) was added into R2. The content was transferred through HLB-AG 11 A8 column and sterile filter to the product vial. R2 was rinsed with 3 mL sterile water (Vial 13) and the solution was transferred to the product vial. The final product, [^{18}F]FGln, was diluted with appropriate volume of sterile saline.



Scheme 1 The synthetic scheme of [^{18}F]FGln

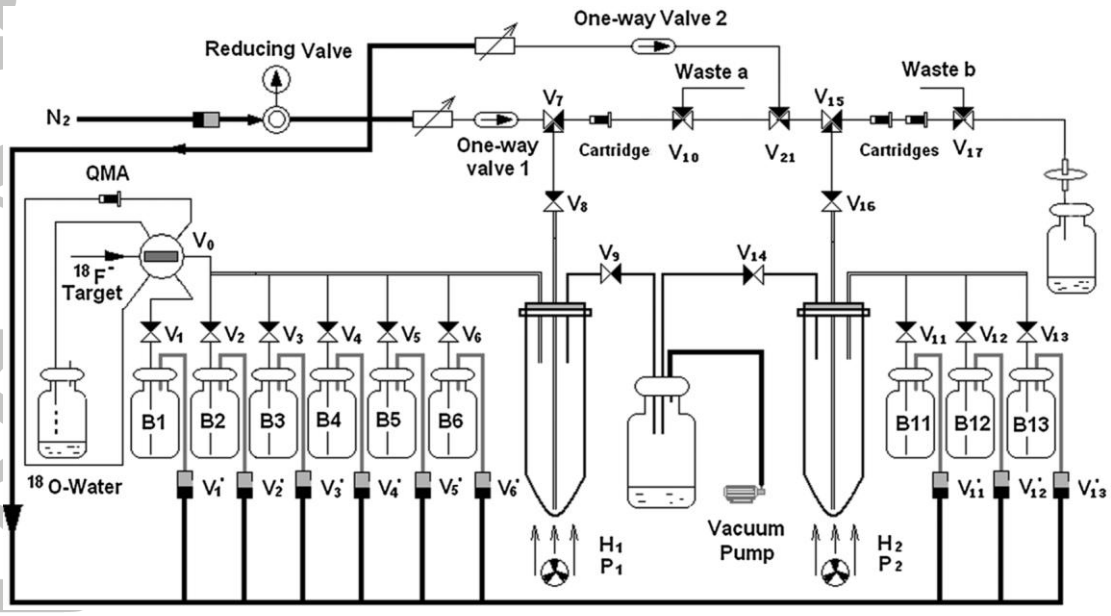


Fig.1 Graphical display of the monitor of [^{18}F]FGln radiosynthesis on the PET-MF-2V-IT-I synthesizer

2.3 Quality control

Quality control tests (requirements of Chinese Pharmacopeia), including appearance, pH, radiochemical identity, radiochemical purity, optical purity, radionuclide identity, residual solvent (acetonitrile, methanol), sterility and bacterial endotoxin, were carried out. Analysis of radiochemical identity and radiochemical purity was performed on an Agilent 1260 Infinity HPLC system equipped with radio and UV detection (254 nm) in series. A chiral column (Chirex 3126 (D)-penicillamine, 250 × 4.6 mm, Phenomenex) was used, with an isocratic method of 1 mM $CuSO_4$, and flow rate 1 mL/min. The optical purity was determined by the same chiral HPLC. The retention time of [^{18}F]FGln was 14.7 min (Fig.2).

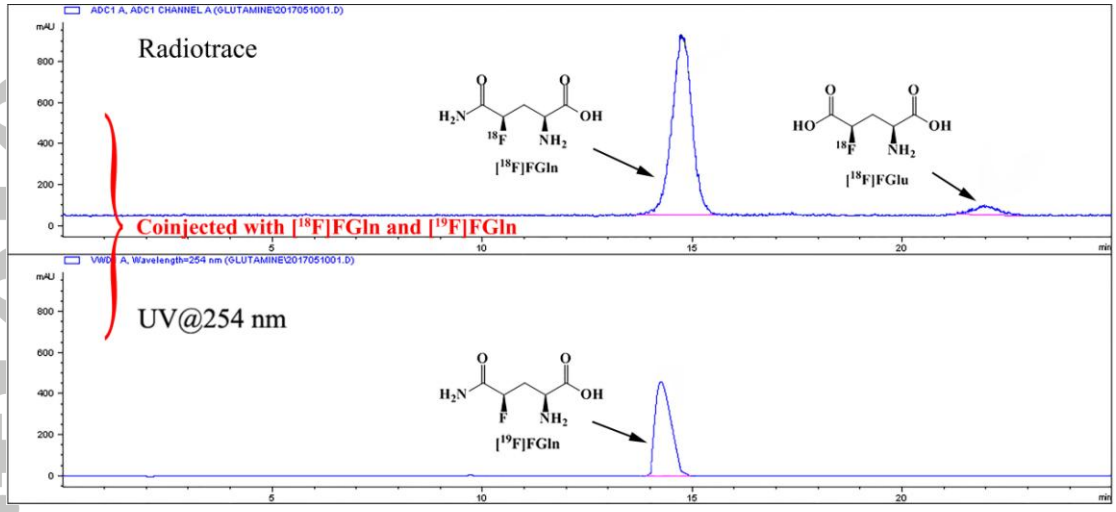


Fig. 2 HPLC profiles of [^{18}F]FGln (Radiotrace), coinjected with[^{19}F]FGln (UV trace) on chiral column (Chirex 3126 (D)-penicillamine, 1mM $CuSO_4$ solution, 1 mL/min)

In addition to the analytical HPLC, quality control tests were performed in accordance with the Chinese Pharmacopoeia for radiopharmaceuticals. Details of the tests performed and the release criteria are given in Table 1.

Table 1 Quality control tests of [^{18}F]FGln

Test	Release Criteria	Method
Appearance	Clear, colorless, and particle-free	Visual inspection
pH	5.0–8.0	pH indicator strip
Radiochemical purity	> 90%	HPLC
Optical purity	> 95%	HPLC
Radiochemical identity	Retention time difference of radioactivity and reference peak ≤ 10 %	HPLC
Chemical impurities (not included [^{19}F]FGln, by UV at 254 nm)	< 5 μg/mL	HPLC
Radionuclide identity	^{18}F ($t_{1/2}$ = 105–115 min)	Measured by dose calibrator
Residual acetonitrile	< 0.041% w/w	GC
Residual methanol	< 0.3% w/w	GC
Sterility	Sterile	Chinese Pharmacopeia
Endotoxin Level	< 5 EU/mL	Chinese Pharmacopeia

2.4 Human PET/CT imaging study

This study was approved by the Ethic Committee of Peking University Cancer Hospital (Permission No. 2017KT38) and conducted according to the latest guidelines of the Declaration of Helsinki. Written informed consent was obtained from each participant prior to PET/CT studies. Patients with specific primary malignant tumor and suspected cerebral metastasis were enrolled in this study. Pathologic analyses of the primary lesions in all patients were performed prior to this clinical trial. One renal cell carcinoma patient recruited for this study was injected with a dose of [^{18}F]FGln (3.7 MBq per kilogram). This patient underwent a static whole-body PET/CT using a Biograph mCT Flow 64 scanner (Siemens, Erlangen, Germany) at 60 min after intravenous injection and the acquisition time was 3 min. PET/CT image of this patient study was presented in Figure3.

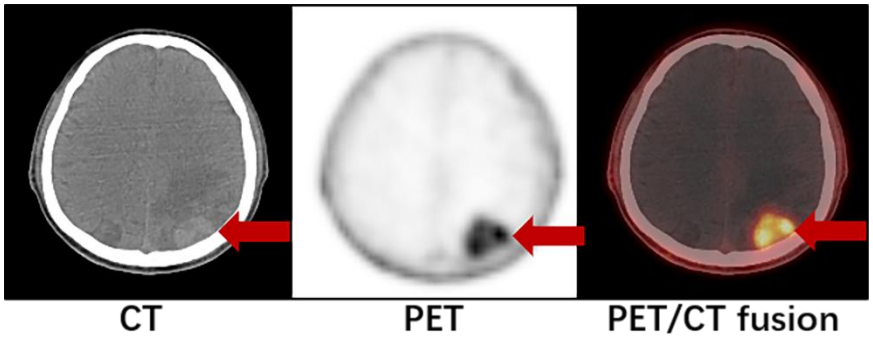


Fig. 3 An axial PET/CT fusion image of [^{18}F]FGln in a 47-y-old man with brain metastasis in renal cell carcinoma (SUVmax=5) after iv injection of [^{18}F]FGln. The image was acquired at 60 min (a sum of 3 min). Similar studies of using [^{18}F]FGln to study metastatic brain tumor has been previously reported.[6]

3. Results and discussion

The two-step automated radiosynthesis of [^{18}F]FGln was performed on a PET-MF-2V-IT-I synthesizer (PET Co. Ltd., Beijing, China, see Fig. 1). The process was validated for each batch of new precursors. The total radiosynthesis time was about 65 min, the decay-corrected radiochemical yield was 18.0 ± 4.2% (n = 59), the radiochemical purity was greater than 90% and the optical purity was greater than 95%. When comparing with previously reported HPLC purification method,[13,14] there was a significant increase in efficiency associated with using the SPE method - by reducing preparation time in synthesis

and reducing product loss. In the past one year and five months, we have performed the procedure for 74 times. There were successful preparations (n = 59), while there were also many failures (n = 15). As shown in Table 2.

Table 2 Synthesis results in the past two years

Year	Total	Successes	Failures	RCY(%)	RCP(%)	Synthesis time
2017	45	37	8	14.8–21.9	> 90%	65 ± 5 min
2018	29	22	7	10.2–19.0	> 90%	65 ± 5 min
Total	74	59	15	18.0 ± 4.2		

RCY, radiochemical yield (decay-corrected); RCP, radiochemical purity.

In quality control tests, the radiochemical purity of the final product was greater than 90%, the optical purity was greater than 95%, and the radiochemical identity was confirmed by chiral HPLC, which was eluted at about 14.7 min. Residual acetonitrile and methanol were assayed by GC, and results were lower than 0.041% and 0.3%. Half-life of the final product was 109 ± 1 min. Chemical impurities were lower than 5 μg/mL. All the necessary parameters such as appearance, sterility, endotoxin content and the pH value of the solution meet the requirements for GMP synthesis. The final product, [^{18}F]FGln, which meets release criteria is transferred to nuclear medicine clinic for patient injection.

Because of water contamination in TFA and instability of the precursor (optical rotation changed), there were often unsuccessful radiosynthesis, the radiochemical purity was lower than 90% and the optical purity was lower than 95%. Failed synthesis also caused by hydrolysis of [^{18}F]FGln to the side product, (2*S*,4*R*)-4-[^{18}F]fluoroglutamic acid ([^{18}F]FGlu).[5] This often happened on days with high humidity. PET/cyclotron facility in the basement of the hospital was damp and soggy, and the moisture was particularly unforgiving. The failures led to abandoning the clinical scans, causing not only inconvenience to patients but also disappointment to clinicians. Careful planning and prior validations will be essential to ensure the success of preparation of [^{18}F]FGln for clinical application.

4. Conclusion

We report successes and failures of synthesizing (2*S*,4*R*)-4-[^{18}F]fluoroglutamine, [^{18}F]FGln, on a fully-automated PET-MF-2V-IT-I synthesizer under GMP-compliant conditions. The experience helps to identify critical steps and pitfalls for preparation of this

clinically useful metabolic probe. This more streamlined procedure may facilitate using [^{18}F]FGln in clinical studies for measuring glutamine metabolism.

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