Preparation of ^{18}F-fluoromisonidazole (^{18}F-FMISO) using ABT Chemistry Production Module

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BACKGROUND

The OUHSC-College of Pharmacy has installed and commissioned the first Biomarker Generator manufactured by ABT Molecular Imaging, Inc. The Biomarker Generator comes with standardized ^{18}F-FDG synthesis, quality control and single-dose dispensing capabilities in a completely automated fashion. The goal of this study was to test the Chemistry Production Module (CPM) for the synthesis of ^{18}F-fluoromisonidazole (^{18}F-FMISO). The single reactor CPM is designed to produce a single dose of certain ^{18}F-radiopharmaceuticals on-demand using a single use Dose Synthesis Card (DSC). The product thus obtained could be purified by reversed-phase high pressure liquid chromatography (HPLC) or solid-phase extraction for use in animal or human studies. The ^{18}F-FMISO was prepared by the synthetic strategy shown in Figure 1.

METHODS

The Biomarker Generator is a 7.5 MeV positive-ion cyclotron coupled with a customized CPM. No-carrier-added ^{18}F-fluoride was obtained through the ^{18}O(p,n)^{18}F nuclear reaction by irradiation (15 - 40 min, 3.5 µA) of a >95% enriched [^{18}O]water target (280 µl). ^{18}F-F- (185-370 MBq) transferred to a reaction vial containing 200 µl of phase-transfer catalyst solution. The solvents were evaporated under a stream of nitrogen at 110°C. Azeotropic drying was repeated twice with 250 µl portions of acetonitrile to generate the anhydrous [K/K222]-^{18}F complex. NITTP precursor [1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol] (10 mg, ABX, Germany) dissolved in anhydrous acetonitrile (600 µl) was transferred to the dried complex and the reaction was allowed to occur at 110°C for 5 min. The product was hydrolyzed by transferring 2 M HCl (1 ml) and heating the mixture at 110°C for 3 min. Finally, the reaction mixture was neutralized by transferring 120 µl of 10 M NaOH. The appearance of the solution at the end of synthesis, decay-corrected radiochemical yield and the radiochemical purity was recorded as shown in Table 1. The entire synthesis was performed using a custom-written script for automated temperature control, solvent transfer, gas bubbling and reaction times.

The HPLC analyses and purifications were performed on a Beckman System Gold HPLC equipped with a Beckman Model 126 pump, 166 absorption detector (254 nm), and a Bioscan Model B-FC-300 radioactivity detector. HPLC solvents consisted of water containing 0.1% trifluoroacetic acid (solvent A) and acetonitrile containing 0.1% trifluoroacetic acid (solvent B). A Sonoma C18 (ES Industries, 10 µm, 100 Å, 4.6 x 250 mm) column was used with a flow rate of 1.5 ml/min. The HPLC gradient system began with an initial solvent composition of 95% A and 5% B for 2 minutes followed by a linear gradient to 50% A and 50% B in 15 minutes.

A total of seven automated runs were carried out to test the initial conditions. These runs were in addition to the innumerable step-by-step optimizations performed initially either manually or in an automated fashion. We obtained the product in all the automated runs (Table 1). The confirmation of the product formation was based on the HPLC retention times of the cold FMISO standard (Figure 2a) and the ^{18}F-FMISO (Figure 2b). The product purified through an SCX cartridge is shown in Figure 2c.

The synthesized and HPLC-purified ^{18}F-FMISO product was tested by PET imaging in a mouse model carrying HCT-116 colon cancer xenograft tumor. For comparison, the same mouse was also imaged with ^{18}F-FDG (Figure 3). About 100 microCi of the radiopharmaceuticals was injected intravenously, and the distribution was allowed for 2 h. PET imaging (10 min) was performed in PET/CT machine from Gamma Medica Ideas (California). The ^{18}F-FMISO and ^{18}F-FDG imaging studies were separated by 24 h.

RESULTS

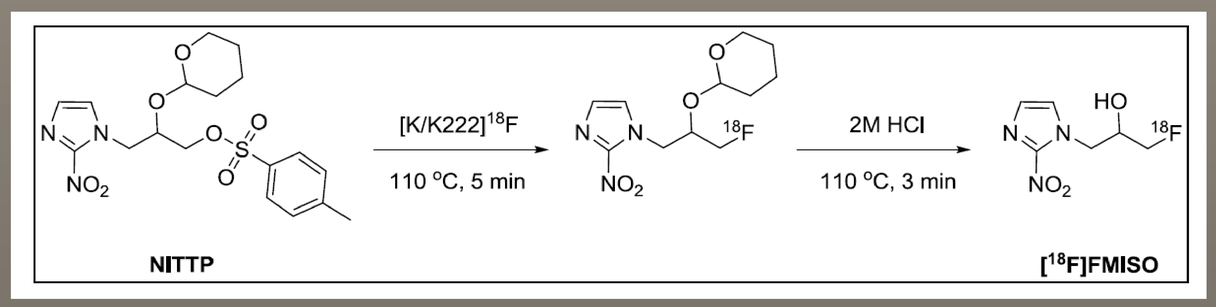

Figure 1: Radiosynthesis of ^{18}F-FMISO. ^{18}F-FMISO was synthesized using standard two step scheme based on NITTP precursor. The final product was neutralized by 10 M NaOH.

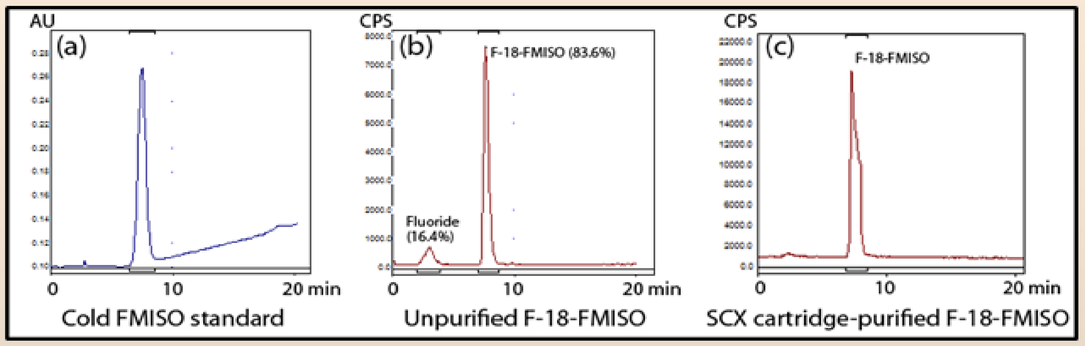

Figure 2: HPLC retention times for (a) standard FMISO, (b) unpurified ^{18}F-FMISO, and (c) ^{18}F-FMISO purified using an SCX cartridge. ^{18}F-FMISO was obtained in high yields without any other degradation side-product. Further purification by either HPLC or an SCX cartridge provides over 95% purity.

Table 1: Synthesis yields of ^{18}F-FMISO using Biomarker Generator

Batch/Recipe	Decay-corrected RCY (%)	RCP (%)	Total Synthesis Time
5/4/12 Batch 1 FMISO V5	46.4	83.6	22 min
5/4/12 Batch 2 FMISO V6	54.5	39.2	22.3 min
5/7/12 Batch 1 FMISO V6a	69.9	86.5	24 min
5/7/12 Batch 2 FMISO V6a	63.2	87.5	22.5 min
5/8/12 Batch 1 FMISO V6a	53.2 (20% decay-corrected HPLC-purified)	100% after HPLC purification	25 min
7/13/12 Batch 1 FMISO V6a	31.3	100% after HPLC purification	24 min
7/13/12 Batch 2 FMISO V6a	48.7	90.8	24 min

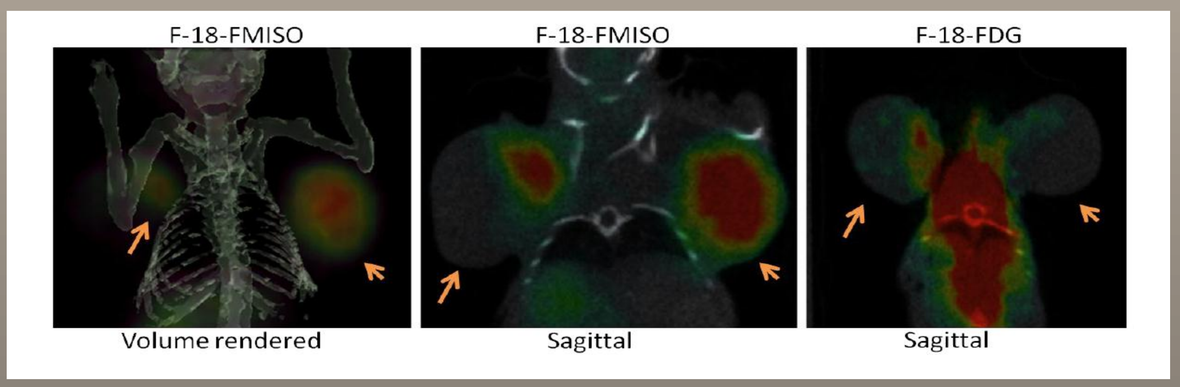

Figure 3: In vivo behavior of ^{18}F-FMISO and ^{18}F-FDG in a mouse carrying xenograft HCT-116 tumor. Clearly, there is a difference in the distribution of the two radiopharmaceuticals in the tumor tissue. Whereas ^{18}F-FMISO accumulated in relatively hypoxic core of the tumor, ^{18}F-FDG was preferentially taken up by metabolically active circumferential tumor tissue..

CONCLUSIONS

Radiosynthesis of ^{18}F-FMISO using ABT's CPM takes about 60-75 min, including HPLC purification. SCX purification is worth pursuing, because it may eliminate the need for HPLC purification. The HPLC-purified ^{18}F-FMISO is obtained in a decay-corrected RCY of approximately 20%. The in vivo behavior of the synthesized ^{18}F-FMISO in tumor tissue was along expected lines.

For more information please visit http://pharmacy.ouhsc.edu/research/imaging.asp or contact vawasthi@ouhsc.edu