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^{18}F–labeled estradiol derivative for targeting estrogen receptor-expressing breast cancer☆,☆☆

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ABSTRACT

Introduction: A novel radiotracer 1 (2 (2 (2 [^{18}F]fluoroethoxy)ethoxy)ethyl) 1H 1,2,3 triazole estradiol ([^{18}F]FETE) was successfully synthesized, characterized and evaluated in mice for estrogen receptor (ER)-positive breast cancer targeting with positron emission tomography (PET) imaging.
Methods: The tosylate precursor **3** was radiolabeled with ^{18}F and then reacted with 17α ethinyl estradiol to produce the final [^{18}F]FETE. The physicochemical properties of [^{18}F]FETE were tested in vitro, including determination of the octanol/water partition coefficient, stability and cellular uptake in MCF-7 (ER-positive) and MDA-MB-231 (ER-negative) cells. An ex vivo biodistribution study was performed in normal Sprague Dawley rats, and in vivo microPET imaging was performed on MCF-7 and MDA-MB-231 tumor-bearing mice. The results of biodistribution and PET imaging of [^{18}F]FETE were compared with that of known 16α [^{18}F] fuoro 17β estradiol ([^{18}F]FES). Radiation dose estimates for [^{18}F]FETE were also analyzed.
Results: [^{18}F]FETE was obtained in high radiochemical yield (46.59 ± 8.06%) with high radiochemical purity (>99%) after HPLC purification and high molar activity (15.45 ± 3.15 GBq/μmol). [^{18}F]FETE is a moderate lipophilic compound with good in vitro stability and the total synthesis time was 55 to 65 min. In biodistribution studies, [^{18}F]FETE showed high uptake in the ER-abundant uterine tissue of normal immature SD rats (8.55 ± 1.21 and 6.83 ± 1.70%ID/g at 1 h after intravenous and intraperitoneal injection, respectively), and could be blocked with estradiol effectively (the uterus uptake was decreased to 0.63 ± 0.35%ID/g at 1 h after iv injection). MicroPET imaging of tumor-bearing mice with [^{18}F]FETE at 1 h after iv injection revealed considerable uptake in ER-positive MCF-7 tumors (4.63 ± 0.73%ID/g) that could be inhibited (1.47 ± 0.29%ID/g) and low uptake in ER-negative MDA-MB-231 tumors (1.97 ± 0.36%ID/g). [^{18}F]FES has relatively low uptake in ER-positive tumor (0.24 ± 0.19%ID/g) when compared with [^{18}F]FETE. The adult female effective radiation dose of [^{18}F]FETE in mice was estimated as 0.0022 mSv/MBq.
Conclusions: A novel 17α ethinyl estradiol-based ER probe [^{18}F]FETE was developed with high molar activity and good in vitro stability. Based on the results of bio-evaluation in normal immature rats and tumor-bearing mice, it might be a promising candidate for specific PET imaging of ER-positive breast cancer.

1. Introduction

Breast cancer is a major health problem for women throughout the world. It has become the second cause of cancer deaths in females [1]. Diagnosis in the early stages is the key to the treatment of breast cancer [2]. According to previous work by Lippman et al. [3] approximately 70–80% of all breast carcinoma are estrogen receptor (ER) positive. Estrogen receptor-positive (ER+) breast tumors are more likely to respond to hormone therapy than ER-negative (ER-) tumors [4]. Knowing the receptor status can lead to more effective individualized treatment.

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With the development of positron emission tomography (PET) and single-photon emission computed tomography (SPECT), many estrogen derivatives and other non-hormonal medicines have been labeled with ^{18}F or other radioisotopes, including ^{11}C, ^{77}Br, ^{99m}Tc, ^{123}I, and ^{125}I, making it possible to quantitatively image in a way that can monitor changes in tumor receptor levels [5].

Of all the ER-imaging agents, 16α [^{18}F]fluoro 17β estradiol ([^{18}F]FES) is the most successful. [^{18}F]FES has good affinity for the ER [6] and is currently in clinical trials as a PET-imaging agent for breast cancer [7]. However, [^{18}F]FES is metabolized mainly in the liver [6], and rapid blood clearance leads to a decrease in specific binding.

In recent years, with the purpose of improving the metabolic stability and enhancing the receptor-binding affinity of [^{18}F]FES for ER imaging, a variety of FES derivatives have been synthesized and evaluated [8–11]. According to previous work, most sites on estradiol, including C-4, C-7, C-11, and C-17, can be substituted and still be tolerated by the ER [8]. Studies of 4 fluoro and 11β methoxy substitutions (4FM [^{18}F]FES) achieved successful PET imaging of a breast cancer mouse model with better sensitivity than [^{18}F]FES [12]. However, 4FM[^{18}F]FES failed to improve on the metabolism of [^{18}F]FES in vivo [12]. PET imaging studies of >20 FES derivatives have been published [8]. However, rapid blood clearance, resulting in low tumor uptake, is still a problem.

Therefore, we designed [^{18}F]FETE, which contains 17α ethinyl estradiol and polyethylene glycol (PEG) moieties. 17α Ethinyl estradiol, with an ethinyl group at C-17, has high affinity for the ER. Its relative binding affinity (RBA) is 190% when the binding affinity of estradiol is set at 100% [13,14]. PEG is a useful addition to lower the lipophilicity of the whole molecule. Theoretically, the addition of PEG could decrease liver metabolism and increase the probability of tumor uptake [10]. In this study, we synthesized and characterized this estradiol-based probe, labeled it with ^{18}F and evaluated its ER-targeting ability in vitro and in vivo.

2. Materials and methods

2.1. Chemistry and radiochemistry

All reagents and solvents were purchased from commercial sources. Reversed-phase high-pressure liquid chromatography (HPLC) was performed on a system with an UltiMate 3000 pump (DIONEX) and a B-FC-1000 flow counter (Bioscan). The C-18 reversed-phase semi-preparative HPLC column (250 * 10 mm, 5-μm particle size [Hypersil GOLD-C18; Thermo Scientific]) was eluted at a flow rate of 4 mL/min.

The syntheses of the [^{18}F]FETE precursor and its nonradioactive reference compound ([^{19}F]FETE) are available in the supplemental data. ^1H NMR spectra were recorded on a Bruker (400 MHz) spectrometer. Mass spectra were obtained with a Bruker Esquire 3000 plus ESI-MS. Chemical shifts are reported in δ (ppm) values.

Fluorine-18 was obtained from the PET Center of the First Affiliated Hospital of Xiamen University. The labeling procedure for [^{18}F]FETE is shown in Scheme 1. [^{18}F]Fluoride was isolated from enriched water by trapping on an anion exchange resin (20 mg) in its carbonate form and then eluting with a CH_3CN-H_2O solution (1 mL, 9:1) of potassium carbonate (3 mg) and Kryptofix [2] (14 mg). This solution was dried by azeotropic distillation with acetonitrile (0.5 mL) three times under a stream of nitrogen at 110 °C, and compound **3** (in 0.2 mL of acetonitrile) was then added. The reaction mixture was shaken at 90 °C for 20 min. After cooling the reaction mixture to room temperature, deionized water (4 mL) was added, and the mixture was passed through a Sep-Pak Plus C18 cartridge, which was then washed with deionized water (4 mL), followed by elution of the [^{18}F]AE intermediate ([^{18}F]-4b in Scheme 1) from the cartridge with 1 mL of tetrahydrofuran (THF). The purity was confirmed by HPLC analysis.

Next, 17α ethinyl estradiol (2 mg), DIPEA (2 mg) and CuI (1 mg) were added to [^{18}F]AE, and the reaction mixture was vibrated at 60 °C for 20 min. Then, the mixture was passed through a syringe filter (0.2 μm). The final product of [^{18}F]FETE was purified by semi-preparative HPLC with a constant gradient (0–20 min) of 35% ACN in pure water (4 mL/min).

[^{18}F]FES was prepared according to the published literature for comparison [9]. The detailed synthetic method is presented in the supplementary information.

2.2. Octanol/water partition coefficient

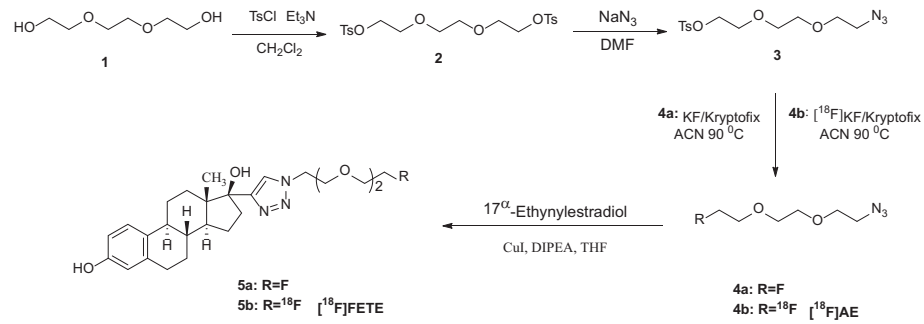
The octanol-to-water partition coefficient of [^{18}F]FETE was measured according to a procedure published previously [15]. Briefly, 1 mL of 1 octanol and 1 mL of saline were added to a centrifuge tube containing 370 kBq of [^{18}F]FETE. The radiotracer was fully mixed with vigorous vortexing for 1 min, and the tube was centrifuged at 5000 rpm for 5 min. Identical volumes of both the organic and inorganic layers were removed and measured in triplicate using a 2480 Wizard [2] Automatic γ-counter (PerkinElmer). Then, the partition coefficient, which is expressed as log P, was calculated.

2.3. In vitro stability of [^{18}F]FETE

The in vitro stability was measured according to a previously published method [16]. Briefly, the radiolabeled compound was incubated in physiological saline at room temperature for 1, 2, and 4 h. To determine its serum stability, the radiotracer was incubated in mice serum at 37 °C for 4 h. To remove plasma proteins from the serum, 1 mL of acetonitrile was added, and the sample was centrifuged. Finally, the radiochemical purities of the radiotracer were analyzed by radio-HPLC using the procedure described above.

2.4. In vivo metabolic stability of [^{18}F]FETE

The metabolic stability studies of [^{18}F]FETE were performed according to published procedures [17,18]. Balb/c mice were intravenously injected with 3.7 MBq of [^{18}F]FETE. The mice were sacrificed at 1 min,



Scheme 1. Synthetic routes for [$^{18/19}F$]FETE.