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^{18}F-labeled rhodamines as potential myocardial perfusion agents: comparison of pharmacokinetic properties of several rhodamines

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ABSTRACT

Introduction: We recently reported the development of the [^{18}F]fluorodiethylene glycol ester of rhodamine B as a potential positron emission tomography (PET) tracer for myocardial perfusion imaging (MPI). This compound was developed by optimizing the ester moiety on the rhodamine B core, and its pharmacokinetic properties were found to be superior to those of the prototype ethyl ester. The goal of the present study was to optimize the rhodamine core while retaining the fluorodiethyleneglycol ester prosthetic group.

Methods: A series of different rhodamine cores (rhodamine 6G, rhodamine 101, and tetramethylrhodamine) were labeled with ^{18}F using the corresponding rhodamine lactones as the precursors and [^{18}F]fluorodiethylene glycol ester as the prosthetic group. The compounds were purified by semipreparative HPLC, and their biodistribution was measured in rats. Additionally, the uptake of the compounds was evaluated in isolated rat cardiomyocytes.

Results: As was the case with the different prosthetic groups, we found that the rhodamine core has a significant effect on the *in vitro* and *in vivo* properties of this series of compounds. Of the rhodamines evaluated to date, the pharmacologic properties of the ^{18}F-labeled diethylene glycol ester of rhodamine 6G are superior to those of the ^{18}F-labeled diethylene glycol esters of rhodamine B, rhodamine 101, and tetramethylrhodamine. As with ^{18}F-labeled rhodamine B, [^{18}F]rhodamine 6G was observed to localize in the mitochondria of isolated rat cardiomyocytes.

Conclusions: Based on these results, the ^{18}F-labeled diethylene glycol ester of rhodamine 6G is the most promising potential PET MPI radiopharmaceutical of those that have evaluated to date, and we are now preparing to carry out first-in-human clinical studies with this compound.

1. Introduction

Cardiovascular disease is a major health problem worldwide. Myocardial perfusion imaging (MPI) using non-invasive modalities such as SPECT (single-photon emission computer tomography) and PET (positron emission tomography) is the most widely used technique for diagnosis and treatment planning in this disease. Currently, the primary method for MPI is SPECT using the single-photon emitters ^{99m}Tc-sestamibi, ^{99m}Tc-tetrofosmin, or ^{201}Tl. However, the limitations of SPECT, including the absence of a standardized attenuation correction method, the inability to perform quantitative measurements, the lower spatial resolution and sensitivity compared to PET as well as recurring shortages of ^{99m}Tc have increased interest in PET MPI [1,2].

Despite the advantages of PET for MPI, its widespread clinical use is hampered by the practical limitations of the currently available PET perfusion agents: [^{13}N]NH_3, [^{15}O]H_2O, and [^{82}Rb]RbCl. The tracers [^{13}N]NH_3 and [^{15}O]H_2O are readily produced by accelerator methods, but the short half-lives of ^{13}N (10 min) and ^{15}O (2 min) restrict their use to clinical centers with an on-site cyclotron. Generator-produced ^{82}Rb ($t_{1/2}$ = 76 s) can be used at clinics without access to cyclotrons, but the high cost of the ^{82}Sr/^{82}Rb generator requires high patient throughput for the generator to be cost effective. Other limiting factors of ^{82}Rb include its less than optimal myocardial extraction and its high positron energy, which results in decreased spatial resolution [1].

The limitations of the existing PET MPI agents and the near ideal physical properties of ^{18}F (ß$^+$, 0.635 MeV [97%]; $t_{1/2}$ = 110 min) have increased interest in the development of ^{18}F-labeled myocardial perfusion tracers. An additional advantage of ^{18}F is that the 110 min half-life is long enough to allow centralized production of ^{18}F-labeled

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radiopharmaceuticals and distribution to clinical centers without an on-site cyclotron, while still being short enough to allow repeated MPI studies of a patient on the same day.

In recent years, several ^{18}F-labeled compounds have been evaluated as possible myocardial perfusion agents including quaternary ammonium salts [3]; tetraphenylphosphonium compounds [4–7]; rotenone derivatives [8,9]; and pyridazinone analogs, such as BMS-747158-02 (Flurpiridaz F18; Lantheus Medical Imaging, Inc.) [10–17]. Flurpiridaz is currently in Phase 3 clinical trials [18–22], and BFPET (Fluoropharma) has recently completed phase 1 clinical trials [7,23].

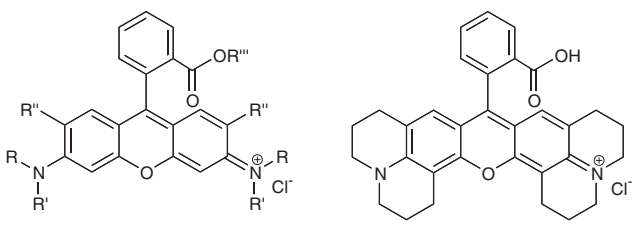
Neither of these ^{18}F-labeled agents has, however, yet been approved by the FDA, and it is not certain that either, or both, will ultimately be approved. Given the clinical importance of MPI, the added clinical value of PET MPI, and the limitations of the existing PET MPI agents, the successful development of an effective ^{18}F-labeled MPI radiopharmaceutical is essential to the care of patients with cardiovascular disease.

We recently reported the development of ^{18}F-labeled esters of rhodamine B as potential MPI radiopharmaceuticals [24,25]. The *in vivo* stability and pharmacokinetics of the ^{18}F-labeled rhodamine B ethyl ester were, however, less than optimal resulting in unfavorable liver uptake. In a study of different rhodamine B esters where we sought to reduce the liver uptake and increase the myocardial uptake of the tracer, we found that the ^{18}F-labeled diethylene glycol ester of rhodamine B ([^{18}F]**2**) was superior to several other rhodamine B esters in terms of *in vivo* stability and pharmacokinetics [26]. In that study we also demonstrated the ability of [^{18}F]**2** to delineate myocardial infarction in a rat [26]. The objective of the present study was to compare the pharmacokinetic properties of several different ^{18}F-labeled rhodamines dyes (i.e., rhodamine 6G, tetramethylrhodamine, and rhodamine 101, Fig. 1) and determine if they provided improved properties compared to [^{18}F]**2**.

2. Methods

2.1. General

Rhodamine 6G chloride was obtained from Acros (Fair Lawn, NJ). Tetramethylrhodamine lactone, rhodamine 101 lactone, and tetrabutylammonium fluoride (1 M in THF) were purchased from Sigma–Aldrich (St. Louis, MO). Diethyleneglycol bistosylate was purchased from TCI America (Philadelphia, PA). For the radiosynthesis, extra dry reagent grade acetonitrile (Thermo Scientific, Bellefonte, PA) and Kryptofix (K 2.2.2, 98%, Sigma–Aldrich) were used. Potassium carbonate (99.97%) was purchased from Alfa Aesar (Ward Hill, MA). Other solvents and reagents were of the highest grade commercially available and used without further purification. Thin-layer chromatography (TLC) was performed using silica gel IB-F coated plastic sheets from J. T. Baker (Phillipsburg, NJ). Nuclear magnetic resonance spectra were obtained using a 400 MHz Varian 400-MR system (Palo Alto, CA). Chemical shifts are given as parts per million (ppm) and are reported relative to tetramethylsilane. Coupling constants are reported in hertz (Hz). The multiplicity of the NMR signals is described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High-resolution mass spectra (ESI-MS mode) were obtained at the University of Illinois Mass Spectrometry Facility using a Micromass 70-VSE spectrometer. Fluorine-18 (as F^- in water) was purchased from Cardinal Healthcare (Woburn, MA) and the Brigham and Women's Hospital BICOR (Boston, MA). The purity of the nonradioactive (^{19}F) reference compounds was ≥95% as determined by analytical HPLC and NMR.



rhodamine B (**2**) R=R'=Et; R''=R'''=H
rhodamine Me4 (**3**) R=R'=Me; R''=R'''=H
rhodamine 6G (**4**) R=R'''=Et; R'=H; R''=Me

rhodamine 101 (**5**)

Fig. 1. Rhodamine dyes discussed in this study.

2.2. Purification and quality control

Analytical HPLC was carried out using a Hitachi 7000 system including an L-7455 diode array detector, an L-7100 pump, and a D-7000 interface. The radiometric HPLC detector was comprised of Canberra nuclear instrumentation modules and was optimized for 511 keV photons. An LaChrom PuroSphere Star C18e column (4 mm × 30 mm, 3 μm) was used for analytical measurements. The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) at a flow rate of 1 mL/min at room temperature. The solvent gradient was 0–15 min (30 — 70% B), 15–25 min (70% B). For semi-preparative HPLC, an ISCO system comprised of an ISCO V4 variable wavelength UV-visible detector (operated at 550 nm), ISCO 2300 HPLC pumps, a radiometric detector similar to that described above, and a Grace Apollo C18 column (10 mm × 250 mm, 5 μm) was used. Semi-preparative HPLC method A (isocratic): 40% solvent A (0.1% TFA in water), 60% solvent B (0.1% TFA in acetonitrile); flow rate, 5 mL/min; room temperature. Semi-preparative HPLC method B (gradient): 0–10 min (40% B); 10–30 min (40 — 50% B); 30–35 min (50 — 100% B); 35–40 min (100% B); flow rate, 5 mL/min; room temperature. Radiofluorination yields were determined by thin-layer chromatography using silica gel plates and chloroform/methanol (8:1 v/v) as the solvent. After development, the TLC strips were cut into 1 cm pieces and counted with a Packard Cobra γ counter.

2.3. Synthesis of non-radiolabeled reference compounds

2.3.1. 2-(2-Fluoroethoxy)ethyl tosylate (1)

Compound **1** was prepared using the previously described procedure [26]. Yield: 262 mg (83%). ^{1}H NMR ($CDCl_3$): δ 7.80 (2H, d, J = 8.26), 7.35 (2H, d, J = 8.20), 4.55 (m, 1H), 4.43(m, 1H), 4.18 (t, 2H, J = 4.80), 3.71 (m, 3H), 3.63 (m, 1H), 2.45 (s, 3H).

2.3.2. Lactone precursors

The rhodamine 6G lactone precursor was prepared as previously described [27] and characterized by ^{1}H NMR and mass spectrometry.

2.3.3. Tetramethylrhodamine 2-(2-fluoroethoxy)ethyl ester, TFA salt (3)

Tetramethylrhodamine lactone (20 mg, 0.05 mmol) was dissolved in 10 mL of acetonitrile, and the solution was heated to 80 °C before adding 40 mg (0.15 mmol) of **1** dissolved in 2 mL of acetonitrile containing 0.1 mL (0.6 mmol) of DIPEA. The mixture was refluxed for 3 h and allowed to cool to room temperature. The condenser was replaced with a serum stopper equipped with a venting needle, and the mixture was heated to 90 °C, allowing the reaction mixture to evaporate slowly to complete dryness (approx. 7 h). HPLC analysis of the reaction mixture revealed incomplete conversion (crude yield 40%). The crude product was dissolved in 3 ml acetonitrile and filtered through a C-18 Sep-Pak cartridge to remove the unreacted lactone, which is retained on the cartridge. In order to obtain high purity reference material, a small fraction of the crude product was further purified by semi-preparative HPLC using the same separation conditions as for the radioactive compound (HPLC method B). Yield (crude): 6 mg (20%). ^{1}H NMR ($CDCl_3$): δ 8.33 (dd, 1H, J = 1.21, 7.84), 7.82–7.73 (m, 2H), 7.33 (dd, 1H, J_1 = 7.34, J_2 = 0.96), 7.10 (d, 2H, J = 9.39), 6.94–6.88 (m, 4H), 4.52 (dt, 2H, J_1 = 47.77, J_2 = 4.03), 3.62 — 3.45 (m, 4H), 3.33 (s, 12H). ^{19} F NMR ($CDCl_3$): −74.7 ppm, (s, 3 F) −222.9 ppm, (m, 1 F). HRMS m/z (%): calcd for $C_{32}H_{38}FN_2O_4^+$ [M^+] 477.2190, found 477.2183 (100%).