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Re(CO)₃([¹⁸F]FEDA), a novel ¹⁸F PET renal tracer: radiosynthesis and preclinical evaluation

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Abstract

Introduction—Our previous work demonstrated that the ^{99m}Tc renal tracer, ^{99m}Tc(CO)₃(FEDA) (^{99m}Tc-1), has a rapid clearance comparable in rats to that of ¹³¹I-OIH, the radioactive gold standard for the measurement of effective renal plasma flow. The uncharged fluoroethyl pendant group of ^{99m}Tc-1 provides a route to the synthesis of a structurally analogous rhenium-tricarbonyl ¹⁸F renal imaging agent, Re(CO)₃([¹⁸F]FEDA) (¹⁸F-1). Our goal was to develop an efficient one-step method for the preparation of ¹⁸F-1 and to compare its pharmacokinetic properties with those of ¹³¹I-OIH in rats.

Methods—¹⁸F-1 was prepared by the nucleophilic ¹⁸F-fluorination of its tosyl precursor. The labeled compound was isolated by HPLC and subsequently evaluated in Sprague-Dawley rats using ¹³¹I-OIH as an internal control and by dynamic PET/CT imaging. Plasma protein binding (PPB) and erythrocyte uptake (RCB) were determined and the urine was analyzed for metabolites.

Results—¹⁸F-1 was efficiently prepared as a single species with high radiochemical purity (>99%) and it displayed high radiochemical stability *in vitro* and *in vivo*. PPB was 87% and RCB was 21%. Biodistribution studies confirmed rapid renal extraction and high specificity for renal excretion, comparable to that of ¹³¹I-OIH, with minimal hepatic/gastrointestinal elimination. The activity in the urine, as a percentage of ¹³¹I-OIH, was 92% and 95% at 10 and 60 min, respectively. All other organs (heart, spleen, lungs) showed a negligible tracer uptake (less than 0.4% ID). Dynamic microPET/CT imaging demonstrated rapid transit of ¹⁸F-1 through the kidneys and into the bladder; there was no demonstrable activity in bone verifying the absence of free [¹⁸F]fluoride.

Conclusions—¹⁸F-1 exhibited a high specificity for the kidney, rapid renal excretion comparable to that of ¹³¹I-OIH and high *in vivo* radiochemical stability. Not only is ¹⁸F-1 a

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promising PET renal tracer, but it provides a route to the development of a pair of analogous $^{18}\text{F}/^{99\text{m}}\text{Tc}$ renal imaging agents with almost identical structures and comparable pharmacokinetic properties. These promising *in vivo* results warrant subsequent evaluation in humans.

Keywords

$\text{Re}(\text{CO})_3([^{18}\text{F}]\text{FEDA})$; radiolabeling; PET imaging; biodistribution; renal radiotracer

1. Introduction

Radionuclide renography has made important contributions to the diagnosis and management of patients with a variety of suspected renal diseases. Chronic kidney disease is as a worldwide public health problem [1, 2] that can be ameliorated by monitoring renal function and detecting specific pathologies at an early stage. The use of $^{99\text{m}}\text{Tc}$ -based radiopharmaceuticals in non-invasive renal scintigraphy has been well documented [3]. Two of the most recognized technetium radiotracers for dynamic planar gamma imaging are $^{99\text{m}}\text{Tc}$ -diethylenetriaminepentaacetic acid ($^{99\text{m}}\text{Tc}$ -DTPA), the only radiopharmaceutical used to measure glomerular filtration rate (GFR) [4], and $^{99\text{m}}\text{Tc}$ -mercaptoacetyltriglycine ($^{99\text{m}}\text{Tc}$ -MAG3), a radiotracer that is primarily cleared by the organic anion transporter 1 (OAT1) located on the basolateral membranes of the proximal tubules [5–7]. $^{99\text{m}}\text{Tc}$ -MAG3 is the radiopharmaceutical of choice for the measurement of effective renal plasma flow (ERPF) [8–11] even if its clearance is only 50%–60% that of ^{131}I -orthiodohippurate (^{131}I -OIH) [12, 13], the clinical radioactive standard for the measurement of ERPF [14]. However, ^{131}I -OIH is no longer commercially available due to the higher radiation exposure and poor imaging characteristics associated with ^{131}I [15].

In the last decade, several new renal radiotracers based on the $^{99\text{m}}\text{Tc}$ -tricarbonyl core have undergone preclinical and clinical evaluation with promising results [16–23]. Among them, $^{99\text{m}}\text{Tc}$ -nitrilotriacetic acid ($^{99\text{m}}\text{Tc}(\text{CO})_3(\text{NTA})$; Figure 1) showed the greatest potential with pharmacokinetic properties in rats [17] and humans [20, 21] comparable to that of ^{131}I -OIH. Because the presence of the charged pendant carboxylate group at physiological pH in the renal tracers has been long considered to be essential for the rapid renal extraction [24], we have recently synthesized three derivatives of $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{NTA})$, $^{99\text{m}}\text{Tc}(\text{CO})_3(2\text{-acetamido})\text{iminodiacetic acid}$ [$^{99\text{m}}\text{Tc}(\text{CO})_3(\text{ADA})$], $^{99\text{m}}\text{Tc}(\text{CO})_3(2\text{-hydroxyethyl})\text{iminodiacetic acid}$ [$^{99\text{m}}\text{Tc}(\text{CO})_3(\text{HDA})$] and $^{99\text{m}}\text{Tc}(\text{CO})_3(2\text{-fluoroethyl})\text{iminodiacetic acid}$ ($^{99\text{m}}\text{Tc}(\text{CO})_3(\text{FEDA})$, **$^{99\text{m}}\text{Tc-1}$**) (Figure 1) and have evaluated these tracers in rats to better assess the importance of a negatively charged uncoordinated group for OAT1 transporter recognition. Even though these three monoanionic $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{NTA})$ analogs lack the negatively charge pendant carboxylate group [22], all three demonstrated high specificity for renal excretion and pharmacokinetics in rats comparable to that of both ^{131}I -OIH and the more hydrophilic and dianionic $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{NTA})$. These results support the conclusion that inner-sphere ligand properties, [$^{99\text{m}}\text{Tc}(\text{CO})_3(\text{N}(\text{CH}_2\text{CO}_2)_2)^-$], shared by all four $^{99\text{m}}\text{Tc}$ -tricarbonyl tracers dominates the properties of the dangling group.

Based on our experience in the use of $^{99m}\text{Tc}/\text{Re}$ -tricarbonyl iminodiacetate systems in the development of renal tubular tracers [16–18, 22, 25–31], we explored the possibility of the introduction of fluorine-18 (^{18}F) radioisotope into the rhenium-tricarbonyl complex (**1**, Scheme 1) in order to develop a new class of positron emission tomography (PET) renal tracers. Labeling the uncharged fluoroethyl group in ^{99m}Tc -**1** (Figure 1) with ^{18}F provides a route to the synthesis of a pair of $^{99m}\text{Tc}/^{18}\text{F}$ renal tracers with almost identical structures that are likely to have similar pharmacokinetic properties. Although PET is increasingly used in early detection and treatment of many diseases, its high spatial resolution, sensitivity, and quantitative accuracy in 3-dimensional tomography make it ideal instrument for functional kidney imaging [32, 33]. Presently there are no PET radiopharmaceuticals comparable to ^{99m}Tc -MAG3 to evaluate the renal function even though several ^{18}F derivatives of ^{131}I -OIH have previously been reported [34, 35]. Moreover, the supply of ^{18}F has the obvious advantage of being unaffected by possible shortages of $^{99m}\text{Mo}/^{99m}\text{Tc}$ generators [36, 37]. Herein, we report on the chemical synthesis, radiolabeling and preclinical evaluation of $\text{Re}(\text{CO})_3(2-[^{18}\text{F}]\text{fluoroethyl})\text{iminodiacetic acid}$ ($\text{Re}(\text{CO})_3([^{18}\text{F}]\text{FEDA}$, **^{18}F -1**), the PET radiolabeled version of ^{99m}Tc -**1**, to determine if it has suitable pharmacokinetic properties for PET kidney imaging.

2. Materials and methods

2.1. General

$\text{Re}(\text{CO})_3(\text{HDA})$ and the non-radioactive reference complex $\text{Re}(\text{CO})_3(\text{FEDA})$ (**1**) were synthesized as previously described [22]. All other reagent-grade chemicals and solvents were obtained from commercial suppliers and used without further purification. ^1H NMR spectra were recorded on a Varian 400 MHz spectrometer, and chemical shifts are reported in δ units using the residual solvent peak as reference; electrospray mass spectrometry (ESI-MS, negative mode) was performed on a Thermo Finnigan LTQ-FT instrument. High performance liquid chromatography (HPLC) analyses of the non-radioactive Re-tricarbonyl complexes (monitored at 254 nm; Waters Breeze system) and of urine metabolites (Beckman Gold Nouveau system) were performed as previously reported [25]. Tissue/organ radioactivity was measured with an automated 2480 Wizard 2 gamma counter (Perkin Elmer) that corrects for spillover from ^{131}I into the ^{99m}Tc window based on a prior normalization process. All animal experiments followed the principles of laboratory animal care and were conducted in compliance with the Emory Institutional Animal Care and Use Committee (IACUC).

2.2. Preparation of $\text{Re}(\text{CO})_3(\text{TsDA})$ (**Re-OTs**; rhenium-tricarbonyl-N-(2-(4-methylbenzenesulphonate)ethyl)iminodiacetic acid)

$\text{Re}(\text{CO})_3(\text{HDA})$ (45 mg, 0.10 mmol) was dissolved in anhydrous acetonitrile and stirred 18 h at room temperature with *p*-toluenesulfonyl chloride (29 mg, 0.15 mmol), triethylamine (21 μL , 0.15 mmol), and a catalytic amount of 4-*N,N*-dimethylaminopyridine (3 mg, 0.02 mmol). The crude product was purified by flash column chromatography on C18 silica gel (Agela Technologist) using water:methanol (50:50). The UV-active fractions were analyzed by HPLC and those without impurities were combined and concentrated to yield the tosylate product **Re-OTs** as a white powder (12 mg, 20%). ^1H NMR (400 MHz, D_2O , pH 7): δ : 7.89

(d, 2H, $J = 8.0$ Hz), 7.54 (d, 2H, $J = 8.0$ Hz), 4.41 (t, 2H), 3.82 (d, 2H, $J = 16$ Hz), 3.72 (t, 2H), 3.49 (d, 2H $J = 16$ Hz), 2.45 (s, 3H). HRMS (M^- , ESI) Calc'd for $C_{16}H_{15}O_{10}N^{187}ReS$: 599.99797, found: 599.99847.

2.3. Synthesis of $Re(CO)_3([^{18}F]FEDA)$ (**^{18}F -1**; rhenium-tricarbonyl-N-(2- $[^{18}F]$ fluoroethyl)iminodiacetic acid)

An aqueous $H^{18}F$ solution (~ 1 Ci), produced by a Siemens 11 MeV RDS 111 cyclotron using the $^{18}O(p,n)^{18}F$ reaction in $H_2^{18}O$, was transferred to a chemical processing control unit (CPCU). It was collected on an ABX QMA trap/release cartridge, released with a solution of K_2CO_3/H_2O (0.6 mL; 1.5 mg/mL), and added to the open CPCU vessel containing a solution of K_{222}/CH_3CN (1.0 mL; 5.0 mg/mL). The $[^{18}F]$ fluoride mixture was dried by azeotropic distillation using additional CH_3CN (3.5 mL) and a combination of heat ($110^\circ C$) and a steady flow of argon to furnish the dry kryptate.

The tosylate precursor **Re-OTs** (3.0 mg) was dissolved in anhydrous CH_3CN (1.0 mL) and added to the conical vessel containing the dry and activated $K_{222}/[^{18}F]$ fluoride complex. The reaction mixture was heated at $110^\circ C$ for 20 min and quenched with the high performance liquid chromatography (HPLC) solvent mixture of 0.05 M triethylammonium phosphate (TEAP) buffer (pH = 7) : abs. EtOH (80 : 20 v/v) (6 mL). The quenched reaction mixture was purified by semi-preparative HPLC using a Waters XTerra Prep RP 18 column (5 μm , 19 $\times$ 100 mm) and eluted with the HPLC solvent at a flow rate of 6 mL/min (isocratic method). The fractions containing **^{18}F -1** were collected from the semi-preparative HPLC and transferred with vacuum into one flask. They were diluted by addition of equivalent volumes of 0.05 M TEAP buffer (pH = 7). After mixing, the homogenous solution was pressure filtered with argon through an Acrodisc filter (pore size 1.0 μm) followed by a smaller filter (pore size 0.2 μm) into a 30 mL vented sterile vial to furnish the final dose solution in 10% EtOH. **^{18}F -1** was prepared in an average decay corrected radiochemical yield (RCY) of 18% (decay corrected from the transfer of $H^{18}F_{(aq)}$ to the CPCU). An aliquot (0.1 mL) of the **^{18}F -1** solution was used to establish its chemical and radiochemical purities by analytical HPLC using a Waters Nova Pak C18 column (4 μm , 3.9 $\times$ 150 mm), a mobile phase of 0.05 M TEAP buffer (pH = 7) : MeOH (80 : 20 v/v) at a flow rate of 1 mL/min (isocratic method). Further evidence for the identity of the radiolabeled product was achieved by co-injection with the authentic standard material $Re(CO)_3(FEDA)$ (**1**) on the analytical HPLC column.

2.4. Biodistribution study

The biodistribution of $Re(CO)_3([^{18}F]FEDA)$ (**^{18}F -1**) was evaluated in normal Sprague-Dawley rats with body weights ranging from 240–284 g by using dual isotope protocol. Rats were anesthetized with ketamine-xylazine (2 mg/kg of body weight) injected intramuscularly, with additional supplemental anesthetic as needed. The bladder was catheterized by use of heat-flared PE-50 tubing (Becton, Dickinson and Co.) for urine collection. Groups of six animals were injected via tail vein with 0.2 mL of a solution containing **^{18}F -1** (100 $\mu Ci/mL$) and ^{131}I -OIH (25 $\mu Ci/mL$) in phosphate-buffered saline (PBS) pH 7.4. One additional aliquot of the ^{18}F and ^{131}I tracer solution (0.2 mL) for each

time point was diluted to 100 mL, and three 1-mL portions of the resulting solution were used as standards.

Animals were sacrificed at 10 min and 60 min after injection. Tissues of interest, along with blood and urine, were collected and placed in counting vials. Each sample and the standards were counted for radioactivity by using an automated gamma-counter using the $^{18}\text{F}/^{131}\text{I}$ dual-label program. The percentage of the dose in each tissue or organ was calculated by dividing the activity counts in each tissue or organ by the total injected activity. The percentage injected dose in whole blood was estimated by assuming a blood volume of 6.5% of total body weight.

Two rats in the 10 min studies produced very little urine (less than 0.2 mL); these rats probably became hypotensive during anesthesia and were eliminated from the combined data analysis.

2.5. microPET imaging

Rat PET/CT imaging was conducted according to the Emory Center for Systems Imaging (CSI) Standard Operating Procedure (SOP). Quantitative whole body rat images were acquired in four Sprague-Dawley rats weighing 223–232 g using a Siemens Inveon MicroPET/CT scanner. The animals were initially anesthetized with ketamine/xylazine mixture injected intramuscularly. An acute intravenous catheter, through which the radiotracer was administered, was placed in the rat's tail vein. Animals were maintained under isoflurane anesthesia on the imaging table using a nose cone apparatus and the vaporizer set to 1–2% at a flow rate of 500–1000 mL/min during the whole experiment. Animals were positioned in the tomograph then fitted with a pulse oximeter min-clip to measure oxygen saturation and heart rate. A rectal probe was used to monitor body temperature, which was maintained by a warm air-filled blanket. A dose of approximately 0.3 mCi of $\text{Re}(\text{CO})_3[^{18}\text{F}]\text{FEDA}$ in 0.2 mL of normal saline was injected via a tail vein. Dynamic PET data were acquired beginning at the time of radiotracer administration for a total duration of 30 min, followed by the acquisition of CT data over a period of 5 min. At the conclusion of the study, the rat was sacrificed prior to regaining consciousness. All data were reconstructed with OSEM3D/MAP using measured attenuation correction derived from the CT. Image data were decay-corrected to the time of injection. The time-activity curves (TAC) of kidneys and other organs (e.g. bladder, heart, liver) were plotted from the appropriate region of interest activities in each time frame. The TAC results were converted to standardized uptake values (SUV) defined by the (activity pixel value in $\mu\text{Ci}/\text{mL}$)*[weight of the animal (g)]/[injected dose (μCi)].

2.6. In vivo stability, plasma protein binding and erythrocyte uptake

The bladders of two additional rats were catheterized as described for the biodistribution study. Both rats received an intravenous tail vein bolus injection of ~0.3 mCi of $\text{Re}(\text{CO})_3[^{18}\text{F}]\text{FEDA}$ and urine was collected for 10 min, filtered with a 0.2 μm Millex-LG filter to remove foreign particles and analyzed by reversed phase HPLC to determine whether the complex was metabolized or excreted unchanged in the urine.

Carotid artery blood samples were obtained and the whole blood samples were placed in capillary tubes and immediately centrifuged for 15 min to determine the hematocrit. Plasma protein binding (PPB) was determined from 1 mL of plasma by ultrafiltration (Centrifree micropartition system; Amicon Inc.) and calculated as $PPB = [1 - (\text{ultrafiltrate concentration}/\text{plasma concentration})] \times 100$. The percent erythrocytes uptake was calculated from the activity counted in the whole blood (counts/g) and packed cells (counts/g) as $[(\text{counts/g in erythrocytes} \times \text{hematocrit})/\text{counts/g in whole blood}]$. No correction was made for plasma trapped in the red blood cells sample. PPB and erythrocytes uptake were calculated in duplicate and the mean values reported. At the conclusion of the study, the rat was sacrificed prior to regaining consciousness.

3. Results and discussion

In this study, we report on the first *in vivo* biological evaluation of $\text{Re}(\text{CO})_3[^{18}\text{F}]\text{FEDA}$ (**^{18}F -1**) as a novel PET imaging agent that is structurally analogous to $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{FEDA})$ (**$^{99\text{m}}\text{Tc}$ -1**). **$^{99\text{m}}\text{Tc}$ -1** is as a promising new $^{99\text{m}}\text{Tc}$ renal tracer with the uncharged fluoroethyl group and with a rapid clearance comparable to that of ^{131}I -OIH [22]. This uncharged fluoroethyl pendant group in **$^{99\text{m}}\text{Tc}$ -1** (Figure 1) provided a means to the synthesis of the structurally analogous **^{18}F -1** tracer. We hypothesized that a pair of $^{18}\text{F}/^{99\text{m}}\text{Tc}$ renal agents with almost identical structures would likely have comparable pharmacokinetic properties.

To test our hypothesis, we had to first develop an efficient one-step labeling method for the preparation of the **^{18}F -1** tracer. We utilized the alcohol moiety of the our recently reported $\text{Re}(\text{CO})_3(\text{HDA})$ complex [27] for the preparation of $\text{Re}(\text{CO})_3(\text{TsDA})$ (**Re-OTs**) as the one-step ^{18}F labelling precursor (Scheme 1, Eq. 2). $\text{Re}(\text{CO})_3(\text{HDA})$ is a rhenium tricarbonyl complex with an iminodiacetate chelate coordinated in an ONO mode around the $\text{Re}(\text{CO})_3$ core forming two five-membered rings and with the dangling *N*-(2-hydroxy)ethyl group (Figure 1) [27]. The novel tosylate **Re-OTs** was obtained by reacting $\text{Re}(\text{CO})_3(\text{HDA})$ with *p*-toluenesulfonyl chloride in anhydrous tetrahydrofuran containing triethylamine and a catalytic amount of 4-*N,N*-dimethylaminopyridine at room temperature. A small aliquot of the reaction mixture was examined by analytical HPLC showing completion of the reaction at 18 h: the starting material was consumed giving rise to a single major product peak with a retention time of 23 min corresponding to the desired tosylate **Re-OTs**. Purification using C18 flash column chromatography yielded the tosylate precursor in 20% yield. Its structure was fully confirmed by spectroscopic and analytical methods (NMR and MS). ^1H NMR spectrum of **Re-OTs** clearly showed two strongly coupled doublets ($J = 16$ Hz, AB-spin system) of the coordinated two acetate moieties verifying no change in an ONO coordination mode with two adjacent 5-membered chelate rings of the iminodiacetate moiety of the starting $\text{Re}(\text{CO})_3(\text{HDA})$ complex. Also, the presence of the singlet signal at 2.45 ppm along with two doublet signals at 7.89 and 7.54 ppm attributed to the methyl group and aromatic protons of the tosyl group, respectively, confirmed the sulfonate formation by conversion of the hydroxyl group of $\text{Re}(\text{CO})_3(\text{HDA})$ into the tosylate in **Re-OTs** (Scheme 1, Eq. 2). Since the ^{18}F labeling is almost always done at higher temperature, the **Re-OTs** precursor was tested for stability at 110 °C for 1 hour and found to be stable at those

conditions as indicated by HPLC as no changes in its chromatogram were observed before and after the heating (see Supporting Information).

Scheme 1 also shows the synthetic routes used to synthesize the non-radioactive standard $\text{Re}(\text{CO})_3(\text{FEDA})$ (**1**). The preparation of **1** from the FEDA ligand is illustrated in Scheme 1, Eq. 1 and was conducted according to our previously reported procedure [22]; this Re complex was used as the reference compound to confirm the chemical identity of ^{18}F -**1**. Furthermore, to test stability of the tosylate precursor in the settings close to ^{18}F radiolabeling conditions, **1** was also obtained following cold fluorination of the tosylate **Re-OTs** with a tetrabutylammonium fluoride (TBAF) solution by heating at 70 °C (Scheme 1, Eq. 2) as confirmed by HPLC. The HPLC chromatogram showed that the peak at 23 min of the starting **Re-OTs** was fully replaced just after 10 min of heating by a major peak at 17 min corresponding to the target compound **1** (see Supporting Information).

$\text{Re}(\text{CO})_3(\text{FEDA})$ was radiolabeled with fluorine-18 at its fluoroethyl moiety from the corresponding tosylate precursor **Re-OTs** using a one-step radiolabeling procedure as outlined in Scheme 2. Fluorination with the cyclotron-produced $[^{18}\text{F}]\text{fluoride}$ as the no-carrier-added activated $\text{K}[^{18}\text{F}]\text{F-Kryptofix 222}$ complex was performed by heating the tosylate **Re-OTs** at 110°C for 20 minutes in anhydrous acetonitrile. The crude radiotracer $\text{Re}(\text{CO})_3([^{18}\text{F}]\text{FEDA})$ (^{18}F -**1**) was purified by semi-preparative HPLC and the desired pure radioactive fractions were combined. The final ^{18}F -**1** radiotracer was formulated in a 0.05 M TEAP solution (pH 7) containing 10% ethanol, collected into a sterile, pyrogen-free product vial after passing through a sterile filter, and was immediately used for biological application. ^{18}F -**1** was obtained in an average decay corrected radiochemical yield of 18% (after the HPLC analysis of the crude product) in a total synthesis time of 120 minutes from end of bombardment. This one-step one pot ^{18}F labeling method can be readily adopted for producing clinical doses of ^{18}F -**1** under cGMP compliance. Since the specific activity of ^{18}F -**1** was not directly determined, the maximum amount of nonradioactive material in the final dose arising from the precursor is about 1 µg. On the basis of an HPLC serial dilution injection of the aqueous solution of $\text{Re}(\text{CO})_3(\text{FEDA})$ (**1**) (100 µg/mL, 10 µg/mL, 1 µg/mL, 0.1 µg/mL, and 0.01 µg/mL; starting from 0.1 µg/mL, no UV peak corresponding to **1** was observed on HPLC chromatogram) using the same analytical HPLC labeling method and a 198 mCi yield in 12 mL solution at end of synthesis, the amount of unlabeled material in the final product would not exceed 0.06 µg/mCi. Quality control HPLC of the formulated ^{18}F -**1** showed chemical and radiochemical purities greater than 99%. The identity of the radiolabeled target compound ^{18}F -**1** was confirmed by co-injection with the standard **1** on analytical HPLC (Figure 2). Both ^{18}F -**1** and **1** complexes showed similar retention times confirming the formation of the same product at the n.c.a (^{18}F -**1**) and macroscopic (**1**) levels. The final solution of the formulated ^{18}F -**1** (pH 7) showed high *in vitro* stability at room temperature. No measurable decomposition was observed when samples of the formulated ^{18}F -**1** solution were analyzed by HPLC over the next 23 hours after the labeling indicating that the ^{18}F -**1** tracer did not release $[^{18}\text{F}]\text{fluoride}$ (Figure 3). To our knowledge, the direct radiofluorination of $\text{Re}(\text{CO})_3([^{18}\text{F}]\text{FEDA})$ is the first example of the incorporation of ^{18}F into a metal-tricarbonyl complex; we initially reported that in our abstract

presentation at the 2016 Society of Nuclear Medicine and Molecular Imaging Annual Meeting [38].

Following the radiosynthesis and chemical and radiochemical characterization of **¹⁸F-1**, we next evaluated the pharmacokinetic properties of this new radiotracer. Biodistribution studies were performed using normal rats and determined at 10 and 60 min post-injection. The pharmacokinetic properties of **¹⁸F-1** were compared to those of ¹³¹I-OIH by simultaneous intravenous administration of both ¹⁸F and ¹³¹I tracers. ¹³¹I-OIH served as an internal controlled since it is the radioactive standard for the measurement of ERPF. The results of the biodistribution studies are shown in Table 1 and are expressed as %ID per organ, blood and urine (bowel includes intestines and stomach). For comparison purposes, Table 1 includes also the previously reported biodistribution data of ^{99m}Tc(CO)₃(FEDA) (**^{99m}Tc-1**), the ^{99m}Tc analog of **¹⁸F-1** [22].

The data obtained for **¹⁸F-1** at 10 and 60 min time points revealed similar biodistributions for **¹⁸F-1** and **^{99m}Tc-1**. Both tracers not only cleared rapidly from the blood (only ~5 % and 0.3 % of injected dose remained in the blood at 10 min and 60 min, respectively) but their blood clearance was also comparable to that of ¹³¹I-OIH at both time points (Table 1). The **¹⁸F-1** tracer demonstrated high specificity for renal excretion. Its urine activity, expressed as a percentage of ¹³¹I-OIH (% ¹⁸F/¹³¹I), was 92 ± 14 % at 10 min and 95 ± 5 % at 60 min, and it was very similar to the results obtained for its **^{99m}Tc-1** analog (% ^{99m}Tc/¹³¹I) (Table 1). The liver activity of **¹⁸F-1** decreased from 5.6 ± 0.3 % ID at 10 min to 0.4 ± 0.1 % ID at 60 min, while uptake in the bowel increased only slightly between these two time points (2.1 ± 1.7 % ID and 3.9 ± 2.0 % ID at 10 and 60 min, respectively), suggesting that the liver activity represented blood pool activity in the liver. Liver and bowel activities of **¹⁸F-1** were analogous to those of **^{99m}Tc-1** and these values were only minimally higher than the liver and bowel activities of ¹³¹I-OIH at the same time points. The heart, lung and spleen all showed a negligible tracer uptake (< 0.4% ID). In addition, bone uptake of **¹⁸F-1** was also negligible at 10 and 60 min (< 0.1% ID/g) verifying the absence of free [¹⁸F]fluoride and supporting the tracer *in vivo* stability.

The biological properties of **¹⁸F-1** in rats appear to be similar to those reported for *p*-¹⁸F-fluorohippuran (¹⁸F-PFH) [34]. They are both exclusively excreted via the renal-urinary pathway with almost identical activities in blood (~ 0.3 % ID) and urine (~ 81% ID) at 1 h post injection. Even though their molecular structures are very different, both of these ¹⁸F renal tracers are likely transported by the same renal organic anion transporter 1 as PAH, ¹³¹I-OIH and ^{99m}Tc-MAG3. One advantage of **¹⁸F-1** over ¹⁸F-PFH is its much simpler one-step one-pot radiolabeling procedure that can better facilitate the **¹⁸F-1** clinical utility. In addition, ¹⁸F-PFH was observed to have a *T*_{1/2} almost twice as great as ¹²⁵I-OIH [39]. The *T*_{1/2} of **¹⁸F-1** was not directly compared with ¹³¹I-OIH in our study but the observation of comparable urine activity at both 10 and 60 minutes suggests that the *T*_{1/2} of **¹⁸F-1** is likely to be similar to that of ¹³¹I-OIH.

The **¹⁸F-1** tracer clearance *via* the renal pathway was also distinctly evident on microPET images. A PET/CT fused image at 30 min after i.v. injection of **¹⁸F-1** in rat is represented in Figure 4. There is no evidence that defluorination occurred *in vivo* since there was no

detectable bone activity (Figure 4). Regions of interest (ROIs) from the whole organs of the kidneys, bladder, heart and liver images were measured so that the tissue accumulation of the radiotracer could be quantified. Dynamic PET imaging revealed the prominent renal uptake with fast excretion into the bladder (Figure 5), confirming that the radioactivity is almost exclusively eliminated by the renal-urinary pathway with only minimal hepatobiliary elimination. Examples of the time-activity curves (TACs) for **¹⁸F-1**, resulting from the subsequent PET data analysis, are shown in Figure 6 for the right kidney, bladder, heart and liver. PET imaging and TACs of **¹⁸F-1** are consistent with the tissue distribution data described above.

To determine *in vivo* stability of **¹⁸F-1**, the tracer was injected into rats and urine was collected for 10 min post-injection. HPLC analysis of urine showed only the intact parent compound **¹⁸F-1** and the absence of any metabolites thus showing that the tracer was excreted unchanged and no defluorination or radioactive degradation of **¹⁸F-1** occurred (Figure 7). Blood from the same rats was obtained to establish the plasma protein binding (PPB) and erythrocyte uptake since both of these parameters can influence the clearance of the renal tracers. In our study, both **¹⁸F-1** and **^{99m}Tc-1** tracers showed similar erythrocyte uptake of 21% vs. 20%, respectively. However, PPB of **¹⁸F-1** was 87% and was higher than PPB of its **^{99m}Tc-1** analog (61%) [22]. Regardless of differences in PPB, however, both **¹⁸F** and **^{99m}Tc** tracers had comparable amounts of injected activity in the urine at 10 and 60 min, and that amount was also comparable to that of **¹³¹I-OIH** (Table 1).

The extraction efficiency of **¹⁸F-1** can be estimated based on the following considerations. The amount of **¹⁸F-1** appearing in the kidney as a function of time is dependent on the renal plasma flow and the extraction efficiency. Renal plasma flow was the same for both **¹⁸F** and **¹³¹I** tracers. The high degree of specificity for the kidney of both **¹⁸F-1** and **¹³¹I-OIH** coupled with their comparable rates of urine excretion (based on the 10 and 60 min time points) indicate that they likely have very similar extraction efficiencies. It is important to note, however, that extraction efficiency is dependent of the affinity of the tracer for the tubular transport mechanism and the availability of the tracer to the transport mechanism. Availability can be modulated by red cell uptake as well as plasma protein binding and plasma protein binding affinity [40]. **¹⁸F-1** has a higher protein binding than **¹³¹I-OIH**, 87% vs 44%, but a lower red cell uptake (21% vs 35%) [17]; the higher plasma protein binding of **¹⁸F-1** may result in diminished access for the tubular transport mechanism compared to **¹³¹I-OIH** resulting in a lower extraction efficiency of **¹⁸F-1** whereas the lower red cell uptake of **¹⁸F-1** compared to **¹³¹I-OIH** may work in the opposite direction. Furthermore, although the higher protein binding of **¹⁸F-1** may result in a diminished availability of **¹⁸F-1** to the tubular transporter, the higher protein binding of **¹⁸F-1** compared to **¹³¹I-OIH** may result in a lower volume of distribution of **¹⁸F-1**, greater retention of **¹⁸F-1** in the plasma and increased availability to the tubular transporter; increased availability could also compensate for a lower extraction efficiency. An accurate determination of the extraction efficiency would require a direct measurement but the more relevant clinical feature is not the extraction efficiency but the rate that the tracer appears in the urine.

4. Conclusions

An efficient, one-step radiosynthesis method was developed for the preparation of **¹⁸F-1**, a new, stable, well-defined ¹⁸F-Re-tricarbonyl PET tracer with an uncharged pendant fluoroethyl group. To our knowledge, the direct radiofluorination of Re(CO)₃[¹⁸F]FEDA is the first example of the incorporation of ¹⁸F into a metal-tricarbonyl complex. **¹⁸F-1** demonstrated high *in vitro* and *in vivo* stability with no metabolic degradation. In addition, **¹⁸F-1** exhibits a high specificity for the kidney and rapid renal excretion comparable to that of ¹³¹I-OIH and its ^{99m}Tc - **1** analog in rats. The results from microPET/CT imaging studies confirm the biodistribution results. These results suggest that **¹⁸F-1** is a promising PET renal tracer that could result in a pair of analogous ¹⁸F/^{99m}Tc renal imaging agents with almost identical structures and pharmacokinetic properties.

The justification of the advantage of having a pair of analogous ¹⁸F/^{99m}Tc renal agents is twofold: (1) We would have an excellent PET tracer comparable to ¹³¹I-OIH; (2) It is often important to determine if a kidney's function is stable, improving or worsening. If one compares sequential ^{99m}Tc-DTPA and ^{99m}Tc-MAG3 studies in a single patient, for example, any difference one observes may reflect the different behavior of the different radiopharmaceuticals, not a change in the patient's renal function; in fact, with two entirely different radiopharmaceuticals, it may be difficult to detect or to be certain of a clinically meaningful change in renal function. We hypothesize that the performance of **¹⁸F-1** will be almost identical to that of ^{99m}Tc-**1**; consequently, we would expect the same results in a given patient whether we used ^{99m}Tc-**1** or **¹⁸F-1**. ^{99m}Tc-**1** is potentially available in kit form and potentially around 5 times cheaper than **¹⁸F-1**. Consequently, most renal studies would likely be performed with ^{99m}Tc-**1**. However, if there is another shortage of ⁹⁹Mo and ^{99m}Tc is not available, the patient could be studied with **¹⁸F-1** and a valid comparison made with the previous ^{99m}Tc-**1** study. Or if the cost of **¹⁸F-1** comes down and PET becomes the imaging procedure of choice, **¹⁸F-1** studies could be compared with the previous ^{99m}Tc-**1** studies.

These promising pre-clinical *in vivo* results warrant further evaluation of **¹⁸F-1** in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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$^{99\text{m}}\text{Tc}(\text{CO})_3(\text{NTA}): \quad \text{R} = \text{CO}_2\text{H}, \quad n = 2$

$^{99\text{m}}\text{Tc}(\text{CO})_3(\text{ADA}): \quad \text{R} = \text{CONH}_2, \quad n = 1$

$^{99\text{m}}\text{Tc}(\text{CO})_3(\text{HDA}): \quad \text{R} = \text{CH}_2\text{OH}, \quad n = 1$

$^{99\text{m}}\text{Tc}(\text{CO})_3(\text{FEDA}): \quad \text{R} = \text{CH}_2\text{F}, \quad n = 1$

Figure 1.

Structures of known $^{99\text{m}}\text{Tc}$ -tricarbonyl renal tracers based on the iminodiacetate chelate with different pendant groups; tracers' overall charges at physiological pH are included.

Figure 2.

HPLC chromatograms of $\text{Re}(\text{CO})_3([^{18}\text{F}]\text{FEDA})$ (**^{18}F -1**; top, red) co-injected with $\text{Re}(\text{CO})_3(\text{FEDA})$ (**1**; bottom, green) to confirm the radiotracer identity (HPLC: 0.05 M TEAP pH 7/MeOH, 80:20 v/v, isocratic method, flow 1 mL/min).

B: 23 hours **A: 2 hours** **Figure 3.**

In vitro stability of $\text{Re}(\text{CO})_3([\text{}^{18}\text{F}]\text{FEDA})$ (¹⁸F-1) at physiological pH was confirmed by HPLC at 2 hours (A) and 23 hours (B) after radiolabeling. The upper trace shows the radio-profile (red) and the lower trace shows the UV profile at 254 nm (green). (HPLC: 0.05 M TEAP pH 7/MeOH, 80:20 v/v, isocratic method, flow 1 mL/min).

Figure 4.
Fused volume rendering of PET and CT (summed 5–30 min) of a normal rat injected with $\text{Re}(\text{CO})_3([\text{}^{18}\text{F}]\text{FEDA})$ ($^{18}\text{F}\text{-1}$) (injected radioactivity: 0.3 mCi, anesthesia: 2% isoflurane).

Figure 5. Representative sequence of microPET maximum intensity projection coronal images (2 min/frame, total 30 min) of $\text{Re}(\text{CO})_3([\text{}^{18}\text{F}]\text{FEDA})$ (^{18}F -1) showing rapid accumulation in the kidneys and clearance into the bladder.

Figure 6. Representative time activity curves (TAC) in kidney, bladder, heart and liver for PET imaging studies with $\text{Re}(\text{CO})_3([^{18}\text{F}]\text{FEDA})$ (^{18}F -1) in rats.

A. Before injection

B. Urine

Figure 7. Radio-HPLC chromatograms of purified $\text{Re}(\text{CO})_3([^{18}\text{F}]\text{FEDA})$ (**^{18}F -1**) before injection (**A**) and of rat urine 10 min after i.v. injection of (**^{18}F -1**) (**B**) (HPLC: 0.05 M TEAP pH 2.5/ MeOH, gradient method [25] flow 1 mL/min).

Eq.1

Eq.2

Scheme 1.

Synthesis of labeling precursor $\text{Re}(\text{CO})_3(\text{TsDA})$ (**Re-OTs**) and reference complex $\text{Re}(\text{CO})_3(\text{FEDA})$ (**1**) starting from the FEDA ligand (Eq.1) [22] and the labeling precursor **Re-OTs** (Eq.2).

Scheme 2.
Radiosynthesis of $\text{Re}(\text{CO})_3([^{18}\text{F}]\text{FEDA})$ (**¹⁸F-1**).

TABLE 1

Comparison of *ex-vivo* biodistribution of $\text{Re}(\text{CO})_3(^{18}\text{F})\text{FEDA}$ (**^{18}F -1**) and $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{FEDA})$ (**$^{99\text{m}}\text{Tc}$ -1**) [^{131}I -OIH (OIH)] at 10 and 60 minutes in normal rats^a. Activity concentrations are expressed as %ID $\pm$ SD in blood, urine and selected organs, and as %ID/g $\pm$ SD in bone for ^{18}F -1.

	10 min		10 min		60 min	
	^{18}F-1	OIH	$^{99\text{m}}\text{Tc}$-1	OIH	^{18}F-1	OIH
Blood	5.2 $\pm$ 0.7	4.5 $\pm$ 0.4	5.1 $\pm$ 0.9	5.8 $\pm$ 1.0	0.3 $\pm$ 0.1	0.8 $\pm$ 0.1
Liver	5.6 $\pm$ 0.3	2.8 $\pm$ 0.6	5.9 $\pm$ 0.8	3.0 $\pm$ 0.9	0.4 $\pm$ 0.1	0.6 $\pm$ 0.1
Bowel ^b	2.1 $\pm$ 1.7	1.3 $\pm$ 0.6	2.9 $\pm$ 0.5	1.8 $\pm$ 0.2	3.9 $\pm$ 2.0	1.1 $\pm$ 0.2
Spleen	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Heart	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Lung	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2	0.4 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
Kidney	9.4 $\pm$ 2.0	5.2 $\pm$ 1.6	6.6 $\pm$ 2.1	4.8 $\pm$ 0.8	1.0 $\pm$ 0.7	1.0 $\pm$ 0.8
Urine	49.3 $\pm$ 3.7	54.4 $\pm$ 6.9	54.1 $\pm$ 7.2	54.2 $\pm$ 9.2	80.5 $\pm$ 4.4	84.8 $\pm$ 3.8
%Urine ^c	92 $\pm$ 14	100 $\pm$ 4			95 $\pm$ 5	99 $\pm$ 2
Bone	0.08 $\pm$ 0.01				0.04 $\pm$ 0.01	

Data are presented as mean $\pm$ SD.

^{a)} **^{18}F -1** (10 min n = 4, 60 min n = 6); **$^{99\text{m}}\text{Tc}$ -1** (10 min n = 5, 60 min n = 4).

^{b)}Bowel includes intestines and stomach.

^{c)}%Urine is expressed as a $^{18}\text{F}/^{131}\text{I}$ -OIH ratio for $\text{Re}(\text{CO})_3(^{18}\text{F})\text{FEDA}$ and $^{99\text{m}}\text{Tc}/^{131}\text{I}$ -OIH ratio for $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{FEDA})$