Published in final edited form as:

Nucl Med Biol. 2014 ; 41(10): 813–824. doi:10.1016/j.nucmedbio.2014.07.009.

Effect of Co-Ligands on Chemical and Biological Properties of $^{99m}\text{Tc(III)}$ Complexes [$^{99m}\text{Tc(L)(CDO)(CDOH)}_2\text{BMe}$] (L = Cl, F, SCN and N₃; CDOH₂ = Cyclohexanedione Dioxime)

Yumin Zheng^{1,2}, Shundong Ji¹, Elena Tomaselli¹, Carley Ernest¹, Tom Freiji¹, and Shuang Liu^{1,*}

¹School of Health Sciences, Purdue University, IN 47907, USA

²Department of Nuclear Medicine, China-Japan Friendship Hospital, Beijing, 100029, China

Abstract

Introduction— ^{99m}Tc -Teboroxime [$^{99m}\text{TcCl(CDO)(CDOH)}_2\text{BMe}$] is a member of the BATO (boronic acid adducts of technetium dioximes) class of $^{99m}\text{Tc(III)}$ complexes. This study sought to explore the impact of co-ligands on solution stability, heart uptake and myocardial retention of [$^{99m}\text{Tc(L)(CDO)(CDOH)}_2\text{BMe}$] (^{99m}Tc -Teboroxime: L = Cl; ^{99m}Tc -Teboroxime(F): L = F; ^{99m}Tc -Teboroxime(SCN): L = SCN; and ^{99m}Tc -Teboroxime(N₃): L = N₃).

Methods—Radiotracers ^{99m}Tc -Teboroxime(L) (L = F, SCN and N₃) were prepared by reacting ^{99m}Tc -Teboroxime with NaF, NaSCN and NaN₃, respectively. Biodistribution and imaging studies were carried out in Sprague-Dawley rats. Image quantification was performed to compare their heart retention and liver clearance kinetics.

Results—Complexes ^{99m}Tc -Teboroxime(L) (L = F, SCN and N₃) were prepared in high yield with high radiochemical purity. All new radiotracers were stable for >6 h in the kit matrix. In its HPLC chromatogram, ^{99m}Tc -Teboroxime showed one peak at ~15.5 min, which was shorter than that of ^{99m}Tc -Teboroxime(F) (~16.4 min). There were two peaks for ^{99m}Tc -Teboroxime(SCN) at 16.5 and 18.3 min. ^{99m}Tc -Teboroxime(N₃) appeared as a single peak at 18.4 min. Their heart retention and liver clearance curves were best fitted to the bi-exponential decay function. The half-

© 2014 Published by Elsevier Inc.

*Correspondence should be addressed to: Dr. Shuang Liu, School of Health Sciences, Purdue University, 550 Stadium Mall Drive, West Lafayette, IN 47907, Phone: 765-494-0236, Fax 765-496-1377, liu100@purdue.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest:

Authors declare that they have no conflict of interest.

Supporting Information Available: HPLC chromatograms of ^{99m}Tc -Teboroxime prepared with excess sodium chloride at T = 0 and 6 h post-labeling (Figure SI1), HPLC chromatograms of ^{99m}Tc -Teboroxime prepared without excess sodium chloride at T = 0 and 6 h post-labeling (Figure SI2), HPLC chromatograms of the reaction solution containing ^{99m}Tc -Teboroxime and excess sodium salt of co-ligands (F⁻, SCN⁻ and N₃⁻) after 2 h reaction at room temperature (Figure SI3), HPLC chromatogram obtained by co-injecting ^{99m}Tc -Teboroxime with ^{99m}Tc -Teboroxime(F), ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N₃) (Figure SI4), HPLC chromatograms of ^{99m}Tc -Teboroxime(F) at T = 0, 2.5 and 6 h post-labeling (Figure SI5), HPLC chromatograms of ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N₃) at 6 h p.i., and selected SPECT images from the SD rats administered with 80 – 90 MBq of ^{99m}Tc -Teboroxime or ^{99m}Tc -Teboroxime(N₃), are all in word document.

times of fast/slow components were $1.6 \pm 0.4/60.7 \pm 8.9$ min for ^{99m}Tc -Teboroxime, $0.8 \pm 0.2/101.7 \pm 20.7$ min for ^{99m}Tc -Teboroxime(F), $1.2 \pm 0.3/84.8 \pm 16.6$ min for ^{99m}Tc -Teboroxime(SCN), and $2.9 \pm 0.9/51.6 \pm 5.0$ min for ^{99m}Tc -Teboroxime(N_3). The 2-min heart uptake followed the order of ^{99m}Tc -Teboroxime ($3.00 \pm 0.37\% \text{ID/g}$) $>$ ^{99m}Tc -Teboroxime(N_3) ($2.66 \pm 0.01\% \text{ID/g}$) $\approx$ ^{99m}Tc -Sestamibi ($2.55 \pm 0.46\% \text{ID/g}$) $>$ ^{99m}Tc -MPO ($2.38 \pm 0.15\% \text{ID/g}$). ^{99m}Tc -Teboroxime remains the best in first-pass extraction. The best image acquisition window is 0 – 5 min for ^{99m}Tc -Teboroxime and 0 – 15 min for ^{99m}Tc -Teboroxime(N_3).

Conclusion—Co-ligands had significant impact on the heart uptake and myocardial retention of complexes [$^{99m}\text{Tc}(\text{L})(\text{CDO})(\text{CDOH})_2\text{BMe}$] ($\text{L} = \text{Cl}, \text{F}, \text{SCN}$ and N_3). Future studies should be directed towards minimizing the liver uptake and radioactivity accumulation in the blood vessels while maintaining their high heart uptake.

Keywords

^{99m}Tc -Teboroxime derivatives; ^{99m}Tc radiotracers; heart imaging

INTRODUCTION

Myocardial perfusion imaging (MPI) with radiotracers is an integral component in evaluation of patients with known or suspected coronary artery disease (CAD) [1-10]. MPI with SPECT (single photon-emission computed tomography) or PET (positron emission tomography) radiotracers remains the only imaging modality available for assessment of physiological consequence of coronary stenosis or myocardial infarction [9]. More than 8-9 million SPECT MPI studies performed in the United States alone 2010 [10]. Both ^{99m}Tc -Sestamibi ($[\text{^{99m}Tc}(\text{MIBI})_6]^+$; MIBI = 2-methoxy-2-methylpropylisonitrile) and ^{99m}Tc -Tetrofosmin ($[\text{^{99m}TcO}_2(\text{tetrofosmin})_2]^+$, tetrofosmin = 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane)) are most widely used radiotracers for MPI [11-18]. The overwhelming success of SPECT MPI is, in large part, attributed to their widespread clinical applications. It is believed that SPECT MPI will continue to play a major role for nuclear cardiology for many years ahead.

A significant drawback of ^{99m}Tc -Sestamibi and ^{99m}Tc -Tetrofosmin is their low first-pass extraction [15-18]. In contrast, ^{99m}Tc -Teboroxime (Figure 1: [$^{99m}\text{TcCl}(\text{CDO})(\text{CDOH})_2\text{BMe}$]; CDOH_2 = cyclohexanedione dioxime), is a member of the BATO (boronic acid adducts of technetium dioximes) class of $^{99m}\text{Tc}(\text{III})$ complexes [19-21], has the highest first-pass extraction fraction among all ^{99m}Tc perfusion radiotracers [22-30]. There is an excellent linear relationship between its heart uptake and blood flow over a wide range (0 – 4.5 mL/min/g) [24,25,27,28]. High first-pass extraction and linear relationship between the blood flow and radiotracer heart uptake permit better detection of the presence and extent of coronary disease, and more precise delineation of perfusion defects, which is of considerable benefit in the management of patients with known or suspected CAD and assessing risk of future cardiac events (e.g. myocardial infarction and sudden death). Despite this advantage, its initial clinical experience was disappointing due to its short myocardial residence time. More than 60% of the initial heart radioactivity is washed out within 5 min post-injection (p.i.) [28-30], which is too fast for standard SPECT cameras to acquire high-quality images. As a result, ^{99m}Tc -Teboroxime was withdrawn from the market even though it was the first

FDA-approved radiotracer for MPI. However, the recent developments in high-speed cardiac SPECT cameras make it possible to use ^{99m}Tc -Teboroxime for SPECT MPI due to its high first-pass extraction fraction [31].

The short myocardial retention of ^{99m}Tc -Teboroxime has been attributed to rapid dissociation of the Cl ligand and its hydrolysis in vivo [19,20]. It was reported that the half-life of Cl^- anion was ~ 13 min under physiological conditions ($\text{pH} = 7.4$) [20]. If this is true, then the myocardial retention of a radiotracer might be improved by increasing its solution stability. With this in mind, we prepared new $^{99m}\text{Tc(III)}$ complexes [$^{99m}\text{Tc(L)}(\text{CDO})(\text{CDOH})_2\text{BMe}$] (^{99m}Tc -Teboroxime(F): $\text{L} = \text{F}$; ^{99m}Tc -Teboroxime(SCN): $\text{L} = \text{SCN}$; and ^{99m}Tc -Teboroxime(N_3): $\text{L} = \text{N}_3$). Thiocyanate and azide anions are used since soft donors (S and N) are expected to form strong bonding with $^{99m}\text{Tc(III)}$. F^- is known to form stable bonds with trivalent metals, such as Al(III) [32-39]. Our hypothesis was that the formation of stable $^{99m}\text{Tc(III)}\text{-L}$ bonds would prevent hydrolysis of $^{99m}\text{Tc(III)}$ radiotracers. In this report, we present the synthesis and evaluation of $^{99m}\text{Tc(III)}$ complexes [$^{99m}\text{Tc(L)}(\text{CDO})(\text{CDOH})_2\text{BMe}$] ($\text{L} = \text{F}$, SCN and N_3) for their potential as heart imaging agents. The objective was to explore the impact of co-ligands on both heart uptake and myocardial retention times of $^{99m}\text{Tc(III)}$ radiotracers. Our long-term goal is to develop a ^{99m}Tc radiotracer with a longer myocardial retention than that of ^{99m}Tc -Teboroxime while maintaining the high heart uptake. The high heart uptake in combination with long myocardial retention makes possible to collect sufficient radioactivity counts using both the standard and ultrafast cardiac SPECT cameras for MPI studies in the future.

EXPERIMENTAL METHODS

Materials

Citric acid, γ -cyclodextrin (γ -CD), cyclohexanedione dioxime (CDOH), diethylenetriaminepentaacetic acid (DTPA), 1,2-diaminopropane- N,N,N',N' -tetraacetic acid (PDTA), methylboronic acid, sodium chloride, sodium fluoride, sodium azide, sodium thiocyanate, and stannous chloride dihydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) were purchased from Sigma/Aldrich (St. Louis, MO), and were used without further purification. $^{99m}\text{TcN-MPO}[\text{[}^{99m}\text{TcN}(\text{mpo})(\text{PNP5})\text{]}^+]$: PNP5 = N-ethoxyethyl-N,N-bis[2-(bis(3-methoxypropyl)phosphino)ethyl]amine) was prepared according to literature methods [40-44], and its radiochemical purity (RCP) was $>95\%$ before being used for biodistribution study. $\text{Na}^{99m}\text{TcO}_4$ were obtained from Cardinal HealthCare® (Chicago, IL). Cardiolite® vials were obtained as a gift from Lantheus Medical Imaging (formerly Bristol Myers Squibb Medical Imaging, N. Billerica, MA). ^{99m}Tc -Sestamibi was prepared according to the manufacturer's package insert.

Radio-HPLC Method

The radio-HPLC method for routine analysis of $^{99m}\text{Tc(III)}$ complexes [$^{99m}\text{Tc(L)}(\text{CDO})(\text{CDOH})_2\text{BMe}$] ($\text{L} = \text{F}$, SCN and N_3) used the Agilent HP-1100 HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a β -ram IN/US detector (Tampa, FL) and Zorbax C₈ column (4.6 mm $\times$ 250 mm, 300 Å pore size; Agilent Technologies, Santa Clara, CA). The flow rate was 1 mL/min. The mobile phase was isocratic with 30% solvent A (10

mM NH₄OAc buffer, pH = 6.8) and 70% solvent B (methanol) between 0 and 5 min, followed by a gradient from 70% solvent B at 5 min to and 90% solvent B at 15 min and continued till 20 min. The instant thin layer chromatography (ITLC) used Gelman Sciences silica-gel strips and a 1:1 mixture of acetone and saline as the mobile phase. ^{99m}Tc(III) complexes and ^{99m}TcO₄⁻ migrated to solvent front while [^{99m}Tc]colloid stayed at the origin. [^{99m}Tc]colloid was reported as the percentage of radioactivity at the origin over the total radioactivity on each strip.

^{99m}Tc-Teboroxime

^{99m}Tc-Teboroxime was prepared using the kit formulation. Each lyophilized vial contains 2 mg of CDOH, 2 mg of methylboronic acid, 50 µg of SnCl₂·2H₂O, 9 mg of citric acid, 2 mg of DTPA, 100 mg of NaCl and 20 mg of γ-cyclodextrin. To a lyophilized vial was added 1.0 mL ^{99m}TcO₄⁻ solution (10 – 30 mCi). The reconstituted vial was then heated at 100 °C for 10 – 15 min. After radiolabeling, the vial was allowed to stand at room temperature for 5 min. A sample of the resulting solution was analyzed by radio-HPLC. The RCP was 95 – 98% with minimal amount of [^{99m}Tc]colloid (<0.5%).

^{99m}Tc-Teboroxime(L) (L = F, SCN and N₃). ^{99m}Tc-Teboroxime was prepared using the procedure above. After radiolabeling, part of the solution (0.3 – 0.5 mL) containing 5 – 15 mCi of ^{99m}Tc-Teboroxime was transferred into a sealed 5 mL vial, which contains sodium fluoride (2 – 5 mg), sodium azide (2 – 3 mg) or sodium thiocyanate (2 – 3 mg) dissolved in dissolved in 0.5 mL of 0.2 M phosphate buffer (pH = 7.4). The reconstituted vial was heated for 5 – 10 min at 100 °C. After the reaction, a sample of the resulting solution was diluted with saline containing ~20% propylene glycol to 500 µCi/mL, and was analyzed by radio-HPLC and ITLC. Their solution stability was monitored by HPLC at 0, 2, 4, and 6 h post-labeling.

Doses Preparation

Doses for biodistribution were prepared by dissolving radiotracer kit solution to ~1.1 MBq/mL with saline containing 20% propylene glycol. Propylene glycol was used to prevent extensive absorption of ^{99m}Tc radiotracers on the surfaces of glass vials or plastic syringe. The injection volume was 0.1 mL for each animal for biodistribution studies. Doses for imaging studies were made by dissolving the ^{99m}Tc radiotracer solution to ~370 MBq/mL with saline containing 20% propylene glycol. All dose solutions were filtered with a 0.20 micron filter unit to eliminate foreign particles before being injected into animals. The injection volume was ~0.1 mL per animal for biodistribution and 0.2 – 0.5 mL per animal for imaging studies.

Animal Preparation

Animal studies were conducted in compliance with the NIH animal experiment guidelines (*Principles of Laboratory Animal Care*, NIH Publication No. 86-23, revised 1985). The protocols for biodistribution and imaging studies were approved by the Purdue University Animal Care and Use Committee (PACUC). The SD rats (200 – 250 g) were purchased from Harlan (Indianapolis, IN), and were acclimated for more than 24 h. Animals were

anesthetized with intramuscular injection of a mixture of ketamine (80 mg/kg) and xylazine (19 mg/kg) before being used for biodistribution and planar imaging studies.

Biodistribution

The SD rats (8 – 12 females and 8 – 12 males) were used for each ^{99m}Tc radiotracer. Each animal was administered with 100 – 111 KBq of ^{99m}Tc radiotracer dissolved in 0.1 mL saline containing 20% propylene glycol via the tail vein. Animals (4 to 6) were sacrificed by sodium pentobarbital overdose (100 – 200 mg/kg) at 2, 15, 30 and 60 min p.i. Blood was withdrawn from the heart. Organs of interest (heart, brain, lungs, liver, spleen, kidneys, muscle and intestines) were excised, rinsed with saline, dried with absorbent tissues, weighed and counted on a Perkin Elmer Wizard – 1480 γ -counter (Shelton, CT). Six extra doses were also weighed and counted before and after organ tissue samples. Organ uptake was calculated and reported as the percentage of injected dose per gram of wet mass (%ID/g).

Planar Imaging

Five SD rats (200 – 250 g) were used for planar imaging. Each animal was administered with ^{99m}Tc radiotracer (50 – 80 MBq) via the tail vein. The animal was placed prone on a single head mini γ -camera (Diagnostic Services Inc., NJ) equipped with a parallel-hole, low-energy, and high-resolution collimator. A standard radiation source was placed beside animal. Static images were acquired at 15, 30 and 60 min p.i. and were stored digitally in a 128×128 matrix. The count limits were set at 300 K. For dynamic imaging during first 5 min, the 1-min static images were acquired, followed by the 2-min static images at 6 – 30, 40, 50 and 60 min p.i.

After imaging, animals were returned to a lead-shielded cage for radiation decay. The images were analyzed by drawing regions of the heart and standard radiation source. The results were corrected for background. The results were expressed as a percentage of the initial activity. The exponential fit of the heart retention and liver clearance were determined using GraphPad Prim 5.0 (GraphPad Software, Inc., San Diego, CA). The imaging quantification data were reported as an average $\pm$ standard deviation on the basis of results from 5 animals in each formulation group.

SPECT Imaging

SPECT images of SD rats ($n = 3$) were obtained using a u-SPECT-II/CT scanner (Milabs, Utrecht, The Netherlands) equipped with a 1.0 mm multi-pinhole collimator. The animal was placed into a shielded chamber connected to an isoflurane anesthesia unit (Univentor, Zejtun, Malta). Anesthesia was induced using an air flow rate of 350 mL/min and ~3.0% isoflurane, and maintained using the air flow rate of ~250 mL/min with ~2.5% isoflurane during the whole time of preparation and image data acquisition (6 frames: 75 projections over 5 min per frame).

The animal was administered with the ^{99m}Tc radiotracer (120 – 180 MBq) in 0.5 mL saline containing ~20% propylene glycol through a catheter, followed with 0.5 mL saline solution flush. Rectangular scan in regions of interest (ROIs) from SPECT and CT were selected one

the basis of the orthogonal X-ray images provided by the CT. After SPECT acquisition, the animal was allowed to recover in a shielded cage.

Image Reconstruction and Data Processing

SPECT reconstruction was performed using a POSEM (pixelated ordered subsets by expectation maximization) algorithm with 6 iterations and 16 subsets. CT data were reconstructed using a cone-beam filtered back-projection algorithm (NRecon v1.6.3, Skyscan). After reconstruction, the SPECT and CT data were automatically co-registered according to the movement of the robotic stage, and then re-sampled to equivalent voxel sizes. Co-registered images were further rendered and visualized using the PMOD software (PMOD Technologies, Zurich, Switzerland). A 3D-Gaussian filter (1.2 mm FWHM) was applied to smooth noise, and the LUTs (look up tables) were adjusted for good visual contrast. The images were visualized as both orthogonal slices and maximum intensity projections.

Data and Statistical Analysis

Biodistribution data, T/B ratios and imaging quantification data were reported as an average $\pm$ standard deviation based on the results from four to six SD rats at each time point. Comparison between two radiotracers was made using a one-way ANOVA test. The level of significance was set at $p < 0.05$.

RESULTS

Synthesis of ^{99m}Tc -Teboroxime(L) (L = Cl, F, SCN and N₃)

^{99m}Tc -Teboroxime was prepared according to the literature method [20,22]. Its RCP is 95 – 98% without purification. Once it was prepared, ^{99m}Tc -Teboroxime was stable for more than 6 h in kit matrix (Figure SI1). In the literature [20,22], excess NaCl (~100 mg per vial) was used to maintain the solution stability of ^{99m}Tc -Teboroxime. We found that NaCl was not needed since the product showed identical HPLC profiles without NaCl (Figure SI2). Complexes ^{99m}Tc -Teboroxime(L) (L = F, SCN and N₃) were prepared (Chart I) by reacting ^{99m}Tc -Teboroxime with NaF, NaSCN and NaN₃, respectively.

The RCP was 85 – 90% for ^{99m}Tc -Teboroxime(F), 90 – 93% for ^{99m}Tc -Teboroxime(SCN), and >95% for ^{99m}Tc -Teboroxime(N₃). The reaction between ^{99m}Tc -Teboroxime and F⁻, SCN⁻ or N₃⁻ was very slow with RCP being <5% after 2 hours of reaction at room temperature (Figure SI3). Heating at 100 °C was required to complete the ligand exchange. The pH value in reaction mixture was controlled using 0.2 M phosphate buffer (pH = 7.4). When the pH was <5.0 in the reaction mixture, the RCP for ^{99m}Tc -Teboroxime(F) and ^{99m}Tc -Teboroxime(SCN) was less than 90%. However, the pH value (5.0 – 8.0) had little impact of the RCP of ^{99m}Tc -Teboroxime(N₃). The optimal amount of NaF was 5 mg/vial while it was ~2 mg/vial for NaSCN and NaN₃.

HPLC Characterization and Solution Stability

Figure 2 shows HPLC chromatograms of ^{99m}Tc -Teboroxime, ^{99m}Tc -Teboroxime(F), ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N₃). There was only one peak at ~15.5 min

for ^{99m}Tc -Teboroxime (Figure 2A). The HPLC retention time of ^{99m}Tc -Teboroxime(F) was ~ 16.4 min (Figure 2B), which was almost 1 min longer than that of ^{99m}Tc -Teboroxime. Co-injection of these two radiotracers further confirmed this observation (Figure SI4). ^{99m}Tc -Teboroxime(SCN) showed two peaks at 16.5 and 18.3 min, likely due to the asymmetric nature of SCN in binding to ^{99m}Tc (III). In contrast, ^{99m}Tc -Teboroxime(N_3) appeared as a single peak at 18.4 min, which was ~ 3 min longer than that of ^{99m}Tc -Teboroxime. It was surprising that ^{99m}Tc -Teboroxime(F) was stable for >6 h at room temperature (Figure SI5). Both ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N_3) remained stable in the kit matrix for more than 6 h at room temperature (Figure SI6).

Dynamic Planar Imaging

Dynamic imaging studies were performed in SD rats. The purpose of these studies was to explore the heart retention and liver clearance rates of ^{99m}Tc radiotracers; and to select appropriate ^{99m}Tc (III) radiotracer(s) for biodistribution. Figure 3 shows the selected planar images of the SD rats administered with ^{99m}Tc -Teboroxime(L) (L = Cl, F, SCN and N_3) over the first 5 min. All images acquired at 0 – 1 min showed high heart and liver uptake; but their lung radioactivity accumulation was low. ^{99m}Tc -Teboroxime(F) showed a lower heart uptake along with a faster heart washout than other three radiotracers. ^{99m}Tc -Teboroxime(N_3) had the longest heart retention among four ^{99m}Tc radiotracers evaluated in this study. Planar image quantification was performed to compare heart retention and liver clearance kinetics of ^{99m}Tc -Teboroxime, ^{99m}Tc -Teboroxime(F), ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N_3) in the SD rats. It was found that their heart retention curves were best fitted to a bi-exponential function (Figure 4). Approximately two-thirds of the heart radioactivity cleared within 5 min for ^{99m}Tc -Teboroxime through the fast component. The half-times of the fast/slow clearance components were calculated to be $1.6 \pm 0.4/60.7 \pm 8.9$ min for ^{99m}Tc -Teboroxime, $0.8 \pm 0.2/101.7 \pm 20.7$ min for ^{99m}Tc -Teboroxime(F), $1.2 \pm 0.3/84.8 \pm 16.6$ min for ^{99m}Tc -Teboroxime(SCN), and $2.9 \pm 0.9/51.6 \pm 5.0$ min for ^{99m}Tc -Teboroxime(N_3). The fast-phase myocardial retention time followed the order of ^{99m}Tc -Teboroxime(N_3) $>$ ^{99m}Tc -Teboroxime $>$ ^{99m}Tc -Teboroxime(SCN) $>$ ^{99m}Tc -Teboroxime(F).

By 20 min p.i., the heart radioactivity almost disappeared for all four radiotracers. The liver clearance curves were also best fitted to the two-phase bi-exponential decay function (Figure 5). Despite their differences in the heart uptake and myocardial retention time, all four radiotracers shared similar liver clearance kinetics with a prolonged liver uptake over the 60-min period (Figure 5). It was interesting to note that there was a significant radioactivity accumulation above the heart (Figure 3) in the SD rats administered with ^{99m}Tc -Teboroxime or ^{99m}Tc -Teboroxime(N_3). The residual time of this radioactivity accumulation was significantly longer in the SD rats administered with ^{99m}Tc -Teboroxime(N_3) than that with ^{99m}Tc -Teboroxime (Figure 3). Another important observation is that ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3) had very little excretion via both renal and hepatobiliary routes during the 60-min study period. Many attempts to collect the urine and feces samples for metabolism studies after planar imaging (60 min p.i.) were made without any success because of the limited amount of radioactivity (<5 μCi collected from each animal).

Biodistribution Properties

The selected biodistribution data and heart/background ratios for ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3) are summarized in Tables 1 and 2, respectively. ^{99m}Tc -Teboroxime(N_3) was of our interest because (1) it exists in solution as a single isomer, (2) its initial heart uptake was close to that of ^{99m}Tc -Teboroxime on the basis of planar imaging, and (3) it had longer myocardial retention than ^{99m}Tc -Teboroxime (Figure 4). The main objective was to compare their heart uptake, myocardial retention and liver clearance kinetics. We found that ^{99m}Tc -Teboroxime(N_3) had significantly lower uptake than ^{99m}Tc -Teboroxime in most organs over the 60 min study period (Tables 1 and 2). For example, ^{99m}Tc -Teboroxime(N_3) showed the blood radioactivity levels of 0.30 ± 0.05 , 0.14 ± 0.01 , 0.13 ± 0.00 and 0.07 ± 0.02 %ID/g at 2, 15, 30 and 60 min p.i., respectively. The blood activity levels for ^{99m}Tc -Teboroxime were 0.47 ± 0.05 , 0.23 ± 0.05 , 0.31 ± 0.09 and 0.22 ± 0.05 %ID/g, respectively, at the same time points. However, the heart uptake of ^{99m}Tc -Teboroxime (3.00 ± 0.37 , 1.25 ± 0.09 , 0.86 ± 0.17 and 0.58 ± 0.04 %ID/g at 2, 15, 30 and 60 min p.i., respectively) was higher than that ^{99m}Tc -Teboroxime(N_3) (2.66 ± 0.01 , 0.90 ± 0.10 , 0.51 ± 0.02 and 0.29 ± 0.02 %ID/g at 2, 15, 30 and 60 min p.i., respectively) over the 60-min period. The initial liver uptake of ^{99m}Tc -Teboroxime(N_3) (3.68 ± 0.09 %ID/g at 2 min p.i.) was slightly higher than that of ^{99m}Tc -Teboroxime (2.87 ± 0.67 %ID/g), but their liver uptake values were almost identical at >15 min p.i., which was consistent with the data from the planar image quantification (Figure 4).

Since the first-pass takes place within the first 2 min after administration of the radiotracer, the higher radiotracer heart uptake at this time point suggests a better first-pass extraction fraction. In this study, we performed biodistribution to compare the 2-min heart uptake of ^{99m}Tc -Teboroxime, ^{99m}Tc -Teboroxime(N_3), ^{99m}Tc -MPO and ^{99m}Tc -Sestamibi in SD rats (Figure 6). ^{99m}Tc -Sestamibi is the most successful radiotracer for SPECT MPI. ^{99m}Tc -MPO is currently under clinical evaluation as a new radiotracer with faster liver clearance kinetics than that ^{99m}Tc -Sestamibi.⁴⁷ It was found that the 2-min heart uptake values followed the general order of ^{99m}Tc -Teboroxime (3.00 ± 0.37 %ID/g) $>$ ^{99m}Tc -Teboroxime(N_3) (2.66 ± 0.01 %ID/g) $\approx$ ^{99m}Tc -Sestamibi (2.55 ± 0.46 %ID/g) $>$ ^{99m}Tc -MPO (2.38 ± 0.15 %ID/g). These results suggested that ^{99m}Tc -Teboroxime remains the best in first-pass extraction fraction.

Radioactivity Accumulation in Blood Vessels

In order to determine where the radioactivity accumulation above the heart was located (Figure 3), we isolated the blood vessels around the heart in SD rats administered with ^{99m}Tc -Teboroxime(N_3). It was found that the uptake was 1.29 ± 0.51 , 1.20 ± 0.67 , 1.10 ± 0.42 and 0.82 ± 0.34 %ID/g at 2, 15, 30 and 60 min p.i., respectively. In fact, the blood vessel uptake was higher than the heart uptake of ^{99m}Tc -Teboroxime(N_3) at >15 min p.i. At this moment, it is not clear why ^{99m}Tc -Teboroxime(N_3) has such a high uptake in the blood vessels. This type of radioactivity accumulation was not seen in SD rats administered with ^{99m}Tc -Sestamibi and ^{99m}Tc -MPO [42-44].

SPECT Imaging

Figure 9A shows the coronal views of SPECT images from the SD rats administered with ^{99m}Tc -Teboroximine or ^{99m}Tc -Teboroximine(N_3). The right and left ventricular walls were clearly delineated. Despite intense liver uptake, as indicated by activity accumulation at the apex of heart, high quality SPECT images could be acquired for both ^{99m}Tc -Teboroximine and ^{99m}Tc -Teboroximine(N_3) due to their high first-pass extraction fractions (Figure 6). We also found that the best image data acquisition window is 0 – 5 min for ^{99m}Tc -Teboroximine (Figure SI7).

Longer acquisition time did not improve the image quality due to its fast myocardial washout and prolonged liver radioactivity accumulation. In contrast, high quality SPECT images was obtained at 0 – 15 min for ^{99m}Tc -Teboroximine(N_3) due to its longer heart retention (Figure SI7). In the sagittal and transaxial SPECT images, there was a significant overlap between the heart and liver activity. This was particularly true in the images obtained at 0 – 15 min (Figure SI7). Figure 7B compares the 3-D SPECT/CT images from the SD rats administered with ^{99m}Tc -Teboroximine or ^{99m}Tc -Teboroximine(N_3) to show the relative location of radioactivity accumulation. The activity in blood vessels was seen in the SD rat administered with ^{99m}Tc -Teboroximine(N_3) (Figure SI7), which is consistent with the biodistribution data (Table 2).

DISCUSSION

Over the last 30 years, ^{99m}Tc -Teboroxime has been believed as a neutral $^{99m}\text{Tc}(\text{III})$ complex with a formula of $[\text{}^{99m}\text{TcCl}(\text{CDO})(\text{CDOH})_2\text{BMe}]$. In solid state, the $\text{Tc}(\text{III})$ center in $[\text{}^{99m}\text{TcCl}(\text{CDO})(\text{CDOH})_2\text{BMe}]$ is indeed seven-coordinated with six imine-N donors and a monodentate chloride in a trigonally-capped prismatic geometry [19,21]. However, its solution structure remains unknown because the chloride ligand is very labile. Considering its extremely low concentration (10^{-8} – 10^{-6} M), it is hard to imagine that the chloride ligand in $[\text{}^{99m}\text{TcCl}(\text{CDO})(\text{CDOH})_2\text{BMe}]$ remains attached the $^{99m}\text{Tc}(\text{III})$ center. Even though excess NaCl (~100 mg per vial or 1.0 – 2.0 M) is often used in the kit formulation to prevent dissociation of chloride ligand, this hardly compares the high concentration of water (~55.6 M). It has been reported that ^{99m}Tc -Teboroxime undergoes rapid the chloro-hydroxy exchange with a half-life of ~13 min under physiological conditions [20]. However, we believe that the actual exchange is between the chloride anion and water. As a result, ^{99m}Tc -Teboroxime most likely exists in solution as its cationic form $[\text{}^{99m}\text{Tc}(\text{H}_2\text{O})(\text{CDO})(\text{CDOH})_2\text{BMe}]^+$ (Chart II). This statement is further supported by the fact that the HPLC retention time of ^{99m}Tc -Teboroxime is ~1 min shorter than that of ^{99m}Tc -Teboroxime(F) (Figure 2B). If ^{99m}Tc -Teboroxime were to exist in solution as its neutral form $[\text{}^{99m}\text{TcCl}(\text{CDO})(\text{CDOH})_2\text{BMe}]$, it would have had the same HPLC retention time as that of ^{99m}Tc -Teboroxime(F) due to the similarity between F^- and Cl^- anions. Because of the rapid equilibrium between $[\text{}^{99m}\text{Tc}(\text{H}_2\text{O})(\text{CDO})(\text{CDOH})_2\text{BMe}]^+$ and $[\text{}^{99m}\text{Tc}(\text{OH})(\text{CDO})(\text{CDOH})_2\text{BMe}]$ (Chart I), ^{99m}Tc -Teboroxime appeared as a single radiometric peak at 15.5 min in its HPLC chromatogram (Figure 2A). If this equilibrium were to be slow, they should have been detected as two separate radiometric peaks due to the difference in their overall molecular charges.

It is well-accepted that the F^- anion is a “hard base”, and the bonding between F^- and metal ions is mainly ionic. It has been reported that the Al(NOTA) (NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid) chelate is an efficient prosthetic group for ^{18}F -labeling of biomolecules [32-39]. Due to the strong binding between F^- and Al(III), the Al(NOTA) ^{18}F chelate remains stable under the physiological conditions. In this study, we were surprised to see that ^{99m}Tc -Teboroxime(F) could be readily prepared in high yield (85 – 90%) in the presence of excess NaCl, its HPLC retention time was about 1 min longer than that of ^{99m}Tc -Teboroxime (Figure 2C), and it was able to maintain its solution stability for more than 6 h in the kit matrix (Figure SI5). If the F^- anion were to be dissociated, ^{99m}Tc -Teboroxime(F) would have shared the same radio-HPLC profile with ^{99m}Tc -Teboroxime under identical chromatographic conditions. Thus, we strongly believe that the F^- anion remains attached to the $^{99m}Tc(III)$ center in solution. It is not clear why the ^{99m}Tc - F bond is so strong, and ^{99m}Tc -Teboroxime(F) is able to maintain its stability in aqueous solution. However, these results suggest that the Tc(III)/Re(III) or Tc(V)-oxo/Re(V)-oxo chelates with appropriate chelators might be useful as prosthetic groups for the ^{18}F -labeling of small biomolecules.

The SCN^- anion is quite unique because it is able to bond to Tc(III) via its both S and N atoms. That might explain why ^{99m}Tc -Teboroxime(SCN) exists in solution as two isomers, as indicated by the presence of two radiometric peaks at 16.5 and 18.4 min in its radio-HPLC chromatogram (Figure 2C). In contrast, N_3^- forms the same complex ^{99m}Tc -Teboroxime(N_3) regardless of which end of N_3^- is attached to $^{99m}Tc(III)$. As a result, ^{99m}Tc -Teboroxime(N_3) shows only one radiometric peak in its HPLC chromatogram (Figure 2D). The radiometric peak at ~18.4 min from ^{99m}Tc -Teboroxime(SCN) is almost identical to that from ^{99m}Tc -Teboroxime(N_3), suggesting that SCN^- bonds to $^{99m}Tc(III)$ mainly via the S-end. Since they have longer HPLC retention times than that of ^{99m}Tc -Teboroxime (Figure 2A), it is reasonable to believe that ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N_3) exist in solution as their neutral forms [$^{99m}Tc(L)(CDO)_2(CDOH)_2BMe$] ($L = SCN$ and N_3) with the $^{99m}Tc(III)$ center being 7-coordinated. This statement is supported by their high solution stability (Figure SI6) due to the capability SCN^- and N_3^- anions to form stronger bonds with $^{99m}Tc(III)$ via both σ - and π -bonding.

Three possible reaction mechanisms (S_N1 , S_N2 and S_N1-CB) have been proposed for the chloro-hydroxide exchange [20]. While ^{99m}Tc -Teboroxime might exist in small portion as its neutral form [$^{99m}TcCl(CDO)_2(CDOH)_2BMe$] in the original vial (pH = 3.0 – 4.0), we believe that the exchange at pH ~ 7.0 is actually between H_2O in [$^{99m}Tc(H_2O)(CDO)(CDOH)_2BMe$] $^+$ and F^- , SCN^- or N_3^- anion. This statement is supported by the fact that increasing the chloride concentration to >1 M does not affect the ligand exchange rate. Chart II shows three possible mechanisms (S_N1 , S_N2 and S_N1-CB) for the ligand exchange between [$^{99m}Tc(H_2O)(CDO)(CDOH)_2BMe$] $^+$ and L ($L = F^-$, SCN^- and N_3^-). In the S_N1 mechanism, the first step is dissociation of H_2O to form the 6-coordinated [$^{99m}Tc(CDO)(CDOH)_2BMe$] $^+$ intermediate, followed by addition of incoming ligand (F^- , SCN^- or N_3^-). However, this mechanism hardly explains the reaction rate dependence on pH, temperature and concentration of F^- , SCN^- and N_3^- anion. The reaction between ^{99m}Tc -Teboroxime and SCN^- was slow at room temperature and the RCP for [$^{99m}Tc(SCN)(CDO)_2(CDOH)_2BMe$]

was <5% (Figure SI2). The S_N2 mechanism involves initial attack by the $F^-/SCN^-/N_3^-$ anion to form an 8-coordinate intermediate, followed by loss of water molecule to afford neutral complexes $[^{99m}Tc(L)(CDO)(CDOH)_2BMe]$ ($L = F, SCN$ and N_3). However, the S_N2 mechanism is not consistent with the pH dependence of ligand exchange. In S_N1 -I-CB mechanism, the rate controlling step is the base-catalyzed removal of a nearby proton preceding elimination of water molecule to form the 6-coordinated intermediate $[^{99m}Tc(L)(CDO)(CDO-H_2O)(CDOH)BMe]$. Since there is no HPLC evidence for the presence of this intermediate, we believe that this intermediate is transient, with the rapid conversion back to a neutral, 7-coordinate complex cation $[^{99m}Tc(H_2O)(CDO)(CDOH)_2BMe]^+$. As illustrated in Chart II, it is possible that the proton eliminated from the BAT backbone during the S_{I-CB} axial ligand exchange process may originate from a free oxime group.

The short myocardial retention times of ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3) are definitely one of their drawbacks when imaging is performed using the standard SPECT cameras, even though ^{99m}Tc -Teboroxime(N_3) has longer heart retention than ^{99m}Tc -Teboroxime (Figure 4). This drawback can be overcome using ultra-fast cardiac SPECT cameras, in which the cadmium-zinc telluride (CZT) solid-state detectors are used to replace traditional NaI(Tl) detectors. The CZT-based cardiac camera allows a more than five-fold reduction in the scan time using same amount of radiotracer and provides clinical information equivalent to that from the standard SPECT MPI [46-52]. The increased sensitivity could also allow the use of lower radiotracer dose to reduce the radiation exposure to patient without loss of image quality. Another drawback of ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3) is their high liver uptake over the 60-min period (Figure 6). High liver radioactivity accumulation may interfere with visualization of the inferior wall of the heart using the standard SPECT cameras. For ultra-fast cardiac SPECT cameras, it is possible to position the patient upright during image acquisition, which would be helpful in minimizing the interference from the liver radioactivity because liver tends to drop downward in this position, allowing better separation of the cardiac and hepatic radioactivity [9,51,52]. As a result of their fast heart washout and prolonged liver radioactivity accumulation, we believe that ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3) are better suited for early image acquisition with ultra-fast cardiac SPECT cameras.

Dynamic planar imaging is an important screening tool to evaluate the heart retention and liver clearance kinetics of ^{99m}Tc radiotracers without sacrificing a large number of animals. Image quantification could be achieved by using an external radiation source with the known amount of radioactivity. While it is possible to calculate the percentage of the injected radioactivity (% ID) in the heart, it is much more difficult to do the same for the liver radioactivity due to its larger size and difficulty to identify the boundary between liver and other organs in the region. Thus, the relative liver radioactivity has to be expressed as the percentage of the initial uptake.

Another important finding of this study is that ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3) show high radioactivity accumulation in blood vessels (Figure 3). This is particularly obvious in the 3D SPECT/CT image of the SD rat administered with ^{99m}Tc -Teboroxime(N_3) (Figure 7B). As a matter of fact, the blood vessel uptake values of ^{99m}Tc -Teboroxime(N_3) is significantly ($p < 0.05$) higher than that in the heart at >15 min p.i.

(Table 2). It is not clear why ^{99m}Tc -Teboroxime(N_3) have such a high uptake in the blood vessels around the heart. This type of radioactivity accumulation has not been seen in the SD rats administered the cationic ^{99m}Tc radiotracers, such as ^{99m}Tc -Sestamibi and ^{99m}Tc -MPO [42-44].

The heart localization mechanism of ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3) is not known. It is also unknown whether they actually enter myocytes or simply attach to phospholipid layers of the cell membrane. It has been suggested that $[\text{}^{99m}\text{Tc}(\text{H}_2\text{O})(\text{CDO})(\text{CDOH})_2\text{BMe}]^+$ is responsible for the high heart uptake of ^{99m}Tc -Teboroxime [20]. This assumption seems consistent with the heart localization mechanism of most cationic ^{99m}Tc and ^{18}F radiotracers [9,53-64], and supported by the low heart uptake and fast heart washout of ^{99m}Tc -Teboroxime(F). However, this hardly explains the fact that ^{99m}Tc -Teboroxime(N_3), also a neutral $^{99m}\text{Tc}(\text{III})$ complex, has a relatively high initial heart uptake (2.66 ± 0.01 %ID/g at 2 min p.i.) with the longer myocardial retention time (Figure 5: $T_{1/2} = 2.9 \pm 0.9$ min) as compared to that of ^{99m}Tc -Teboroxime (Figure 5: $T_{1/2} = 1.6 \pm 0.4$ min). Therefore, there must be an alternative mechanism for the high heart uptake of ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3). Like ^{99m}Tc -NOET ($[\text{}^{99m}\text{Tc}(\text{NOET})_2$: NOET = N-ethoxy-N-ethyldithiocarbamate) [65-68], ^{99m}Tc -Teboroxime(N_3) may bind to the L-type calcium channels in the open configuration without entering myocytes, its cellular uptake mechanism is not energy-dependent. It must be noted that this explanation remains largely speculation in the absence of more experimental data.

CONCLUSIONS

In this study, we found that all new radiotracers $[\text{}^{99m}\text{Tc}(\text{L})(\text{CDO})_2(\text{CDOH})_2\text{BMe}]$ ($\text{L} = \text{F}$, SCN and N_3) are neutral in their overall molecular charge and remain stable in the reaction mixture for >6 h. The co-ligands in $[\text{}^{99m}\text{Tc}(\text{L})(\text{CDO})(\text{CDOH})_2\text{BMe}]$ ($\text{L} = \text{Cl}$, F , SCN and N_3) had significant impact on their solution stability, heart uptake and myocardial retention. ^{99m}Tc -Teboroxime(N_3) had a longer myocardial retention than ^{99m}Tc -Teboroxime despite of its relatively lower heart uptake. The results from SPECT studies suggest that the best image data acquisition window is 0 – 5 min for ^{99m}Tc -Teboroxime, and 0 – 15 min for ^{99m}Tc -Teboroxime(N_3) due to its longer myocardial retention. Future studies should be directed towards minimizing the liver uptake and radioactivity accumulation in blood vessels while maintaining their high heart uptake.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This work was supported, in part, by Purdue University, the Indiana Clinical and Translational Sciences Institute funded in part by grant Number TR000006 (Clinical and Translational Award) from the National Institutes of Health, the National Center for advancing Translational Science, and R21 EB017237-01 (S.L.) from the National Institute of Biomedical Imaging and Bioengineering (NIBIB).

ABBREVIATIONS

BATO	boronic acid adducts of technetium dioximes
CAD	coronary artery disease
DTPA	diethylenetriaminepentaacetic acid (or pentetic acid)
MPI	myocardial perfusion imaging
RCP	radiochemical purity
SPECT	single photon-emission computed tomography
^{99m}TcN-MPO	[^{99m} TcN(mpo)(PNP5)] ⁺ (mpo = 2-mercaptopyridine oxide, and PNP5 = N-ethoxyethyl-N,N-bis[2-(bis(3-methoxypropyl)phosphino)ethyl]amine)
^{99m}TcN-NOET	^{99m} TcN(NOET) ₂ (NOET = N-ethoxy-N-ethylthiocarbamate)
^{99m}Tc-Sestamibi	[^{99m} Tc(MIBI) ₆] ⁺ (MIBI = 2-methoxy-2-methylpropylisonitrile)
^{99m}Tc-Tetrofosmin	[^{99m} TcO ₂ (tetrofosmin) ₂] ⁺ (tetrofosmin = 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane)
^{99m}Tc-Teboroxime	[^{99m} TcCl(CDO)(CDOH) ₂ BMe] (CDOH ₂ = cyclohexanedione dioxime)
^{99m}Tc-Teboroxime(F)	[^{99m} Tc(F)(CDO)(CDOH) ₂ BMe]
^{99m}Tc-Teboroxime(SCN)	[^{99m} Tc(SCN)(CDO)(CDOH) ₂ BMe]
^{99m}Tc-Teboroxime(N₃)	[^{99m} Tc(N ₃)(CDO)(CDOH) ₂ BMe]

References

1. Acampa W, Di Benedetto C, Cuocolo A. An overview of radiotracers in nuclear cardiology. *J Nucl Cardiol*. 2000; 7:701–7. [PubMed: 11144485]
2. Dilsizian V. The role of myocardial perfusion imaging in vascular endothelial dysfunction. *J Nucl Cardiol*. 2000; 7:180–4. [PubMed: 10796009]
3. Beller GA, Zaret BL. Contributions of nuclear cardiology to diagnosis and prognosis of patients with coronary artery disease. *Circulation*. 2000; 101:1465–8. [PubMed: 10736294]
4. Parker JA. Cardiac nuclear medicine in monitoring patients with coronary heart disease. *Semin Nucl Med*. 2001; 31:223–7. [PubMed: 11430529]
5. Kapur A, Latus KA, Davies G, Dhawan RT, Eastick S, Jarritt PH, et al. A comparison of three radionuclide myocardial perfusion tracers in clinical practice: the ROBUST study. *Eur J Nucl Med Mol Imaging*. 2002; 29:1608–16. [PubMed: 12458395]
6. Henneman MM, Schuijf JD, van der Wall EE, Bax JJ. Non-invasive anatomical and functional imaging for the detection of coronary artery disease. *Br Med Bull*. 2006; 79-80:187–202. [PubMed: 17182724]
7. Salerno M, Beller GA. Noninvasive assessment of myocardial perfusion. *Circ Cardiovasc Imaging*. 2009; 2:412–24. [PubMed: 19808630]

8. Stirrup J, Wechalekar K, Maenhout A, Anagnostopoulos C. Cardiac radionuclide imaging in stable coronary artery disease and acute coronary syndromes. *Br Med Bull.* 2009; 89:63–78. [PubMed: 19179343]
9. Gaemperli O, Kaufmann PA. Lower dose and shorter acquisition: pushing the boundaries of myocardial perfusion SPECT. *J Nucl Cardiol.* 2011; 18:830–2. [PubMed: 21681614]
10. Perrone-Filardi P, Costanzo P, Dellegrottaglie S, Gargiulo P, Ruggiero D, Savarese G, et al. Prognostic role of myocardial single photon emission computed tomography in the elderly. *J Nucl Cardiol.* 2010; 17:310–5. [PubMed: 20033857]
11. Nunn AD. Radiopharmaceuticals for imaging myocardial perfusion. *Semin Nucl Med.* 1990; 20:111–8. [PubMed: 2184519]
12. Saha GB, Go RT, MacIntyre WJ. Radiopharmaceuticals for cardiovascular imaging. *Int J Rad Appl Instrum B.* 1992; 19:1–20. [PubMed: 1577608]
13. Opie LH, Hesse B. Radionuclide tracers in the evaluation of resting myocardial ischemia and viability. *Eur J Nucl Med.* 1997; 24:1183–93. [PubMed: 9283116]
14. Jain D. Technetium-99m labeled myocardial perfusion imaging agents. *Semin Nucl Med.* 1999; 29:221–36. [PubMed: 10433338]
15. Banerjee S, Pillai MR, Ramamoorthy N. Evolution of Tc-99m in diagnostic radiopharmaceuticals. *Semin Nucl Med.* 2001; 31:260–77. [PubMed: 11710769]
16. Kailasnath P, Sinusas AJ. Comparison of Tl-201 with Tc-99m-labeled myocardial perfusion agents: technical, physiologic, and clinical issues. *J Nucl Cardiol.* 2001; 8:482–98. [PubMed: 11481571]
17. Llauro JG. The quest for the perfect myocardial perfusion indicator...still a long way to go. *J Nucl Med.* 2001; 42:282–4. [PubMed: 11216527]
18. Baggish AL, Boucher CA. Radiopharmaceutical agents for myocardial perfusion imaging. *Circulation.* 2008; 118:1668–74. [PubMed: 18852377]
19. Treher EN, Francesconi LC, Gougoutas JZ, Malley MF, Nunn AD. Monocapped Tris(dioxime) complexes of technetium(III): synthesis and structural characterization of $\text{TcX}(\text{dioxime})_3\text{B-R}$ ($\text{X} = \text{Cl, Br}$; dioxime = dimethylglyoxime, cyclohexanedione dioxime; $\text{R} = \text{CH}_3, \text{C}_4\text{H}_9$). *Inorg Chem.* 1989; 28:3411–6.
20. Jurisson SS, Hirth W, Linder KE, Di Rocco RJ, Narra RK, Nowotnik DP, et al. Chloro $\rightarrow$ hydroxy substitution on technetium BATO $[\text{TcCl}(\text{dioxime})_3\text{BR}]$ complexes. *Int J Rad Appl Instrum B.* 1991; 18:735–44. [PubMed: 1787082]
21. Jurisson SS, Francesconi L, Linder KE, Treher E, Malley MF, Gougoutas JZ, et al. Synthesis, characterization, and reactivity of manganese and rhenium dioxime complexes. X-ray crystal structures of $[\text{Mn}^{\text{II}}(\text{CDO})(\text{CDOH})_2(\text{BPh}(\text{OCH}_3)_2)_2]$, an unusual pseudocathrochelate complex $[\text{Re}^{\text{III}}\text{Cl}(\text{CDO})(\text{CDOH})_2\text{BPh}]$. *Inorg Chem.* 1991; 30:1820–7.
22. Seldin DW, Johnson LL, Blood DK, Muschel MJ, Smith KF, Wall RM, et al. Myocardial perfusion imaging with technetium-99m SQ30217: comparison with thallium-201 and coronary anatomy. *J Nucl Med.* 1989; 30:312–319. [PubMed: 2738660]
23. Leppo JA, Meerdink DJ. Comparative myocardial extraction of two technetium-labeled BATO derivatives (SQ30217, SQ30214) and thallium. *J Nucl Med.* 1990; 31:67–74. [PubMed: 2295943]
24. Marshall RC, Leidholdt EM Jr, Zhang DY, Barnett CA. The effect of flow on technetium-99m-teboroxime (SQ30217) and thallium-201 extraction and retention in rabbit heart. *J Nucl Med.* 1991; 32:1979–88. [PubMed: 1919742]
25. McSherry BA. Technetium-99m-Teboroxime: a new agent for myocardial perfusion imaging. *J Nucl Med Technol.* 1991; 19:22–6.
26. Iskandrian AS, Heo J, Nguyen T, Mercurio J. Myocardial imaging with Tc-99m teboroxime: technique and initial results. *Am Heart J.* 1991; 121:889–94. [PubMed: 2000757]
27. Fleming RM, Kirkeeide RL, Taegtmeier H, Adyanthaya A, Cassidy DB, Goldstein RA. Comparison of technetium-99m teboroxime tomography with automated quantitative coronary arteriography and thallium-201 tomographic imaging. *J Am Coll Cardiol.* 1991; 17:1297–302. [PubMed: 2016447]
28. Rumsey WL, Rosenspire KC, Nunn AD. Myocardial extraction of teboroxime: effects of teboroxime interaction with blood. *J. Nucl. Med.* 1992(33):94–101. [PubMed: 1530970]

29. Williams KA, Taillon LA, Draho JM, Foisy MF. First-pass radionuclide angiographic studies of left ventricular function with technetium-99m-teboroxime, technetium-99m-sestamibi and technetium-99m-DTPA. *J Nucl Med*. 1993; 34:394–9. [PubMed: 8382741]
30. Johnson LL. Myocardial perfusion imaging with technetium-99m-teboroxime. *J Nucl Med*. 1994; 35:689–92. [PubMed: 8151395]
31. Bailey DL, Willowson KP. An evidence-based review of quantitative SPECT imaging and potential clinical applications. *J Nucl Med*. 2013; 54:83–89. [PubMed: 23283563]
32. McBride WJ, Sharkey RM, Karacay H, D'Souza CA, Rossi EA, Laverman P, et al. A novel method of ^{18}F radiolabeling for PET. *J Nucl Med*. 2009; 50:991–8. [PubMed: 19443594]
33. Laverman P, McBride WJ, Sharkey RM, Eek A, Joosten L, et al. A novel facile method of labeling octreotide with ^{18}F -fluorine. *J Nucl Med*. 2010; 51:454–61. [PubMed: 20150268]
34. Liu S, Liu H, Jiang H, Xu Y, Zhang H, Cheng Z. One-step radiosynthesis of ^{18}F -AlF-NOTA-RGD₂ for tumor angiogenesis PET imaging. *Eur J Nucl Med Mol Imaging*. 2011; 38:1732–41. [PubMed: 21617974]
35. D'Souza CA, McBride WJ, Sharkey RM, Todaro LJ, Goldenberg DM. High-yielding aqueous ^{18}F -labeling of peptides via Al ^{18}F chelation. *Bioconjugate Chem*. 2011; 22:1793–1803.
36. McBride WJ, D'Souza CA, Sharkey RM, Karacay H, Rossi EA, Chang CH, et al. Improved ^{18}F labeling of peptides with a fluoride-aluminum-chelate complex. *Bioconjugate Chem*. 2010; 21:1331–40.
37. Lang L, Li W, Guo N, Ma Y, Zhu L, Kiesewetter DO, et al. Comparison study of [^{18}F]FAI-NOTA-PRGD₂, [^{18}F]FPPRGD₂, and [^{68}Ga]Ga-NOTA-PRGD₂ for PET imaging of U87MG tumors in mice. *Bioconjugate Chem*. 2011; 22:2415–22.
38. McBride WJ, D'Souza CA, Karacay H, Sharkey RM, Goldenberg DM. New lyophilized kit for rapid radiofluorination of peptides. *Bioconjugate Chem*. 2012; 23:538–47.
39. Laverman P, D'Souza CA, Eek A, McBride WJ, Sharkey RM, Oyen WJ, et al. Optimized labeling of NOTA-conjugated octreotide with F-18. *Tumor Biol*. 2012; 33:427–34.
40. Liu S, He Z, Hsieh WY, Kim YS. Evaluation of novel cationic $^{99\text{m}}\text{Tc}$ -nitrido complexes as radiopharmaceuticals for heart imaging: improving liver clearance with crown ether groups. *Nucl Med Biol*. 2006; 33:419–32. [PubMed: 16631092]
41. Kim YS, He Z, Hsieh WY, Liu S. Impact of bidentate chelators on lipophilicity, stability, and biodistribution characteristics of cationic $^{99\text{m}}\text{Tc}$ -nitrido complexes. *Bioconjugate Chem*. 2007; 18:929–36.
42. Kim YS, Wang J, Broisat A, Glover DK, Liu S. Tc-99m-N-MPO: novel cationic Tc-99m radiotracer for myocardial perfusion imaging. *J Nucl Cardiol*. 2008; 15:535–46. [PubMed: 18674722]
43. Kim YS, Shi J, Zhai S, Hou G, Liu S. Mechanism for myocardial localization and rapid liver clearance of Tc-99m-N-MPO: a new perfusion radiotracer for heart imaging. *J Nucl Cardiol*. 2009; 16:571–9. [PubMed: 19288164]
44. Bu L, Li R, Jin Z, Wen X, Liu S, Yang B, et al. Evaluation of $^{99\text{m}}\text{Tc}$ -N-MPO as a new myocardial perfusion imaging agent in normal dogs and in an acute myocardial infarction canine model: comparison with $^{99\text{m}}\text{Tc}$ -Sestamibi. *Mol Imaging Biol*. 2011; 13:121–7. [PubMed: 20458635]
45. Gao S, Zhao G, Wen Q, Bai L, Chen B, Ji T, et al. Pharmacokinetics and biodistribution of $^{99\text{m}}\text{Tc}$ -N-MPO in healthy human volunteers. *Clin Nucl Med*. 2014; 39:e14–e19. [PubMed: 23917788]
46. Kaufmann PA, Gaemperli O. Combining CT and nuclear: a winning hybrid team. *J Nucl Cardiol*. 2009; 16:170–2. [PubMed: 19127395]
47. Esteves FP, Raggi P, Folks RD, Keidar Z, Askew JW, Rispler S, et al. Novel solid-state-detector dedicated cardiac camera for fast myocardial perfusion imaging: multicenter comparison with standard dual detector cameras. *J Nucl Cardiol*. 2009; 16:927–34. [PubMed: 19688410]
48. Buechel RR, Pazhenkottil AP, Herzog BA, Husmann L, Nkoulou RN, Burger IA, et al. Real-time breath-hold triggering of myocardial perfusion imaging with a novel cadmium-zinc-telluride detector gamma camera. *Eur J Nucl Med Mol Imaging*. 2010; 37:1903–8. [PubMed: 20517605]
49. Duvall WL, Croft LB, Godiwala T, Ginsberg E, George T, Henzlova MJ. Reduced isotope dose with rapid SPECT MPI imaging: initial experience with a CZT SPECT camera. *J Nucl Cardiol*. 2010; 17:1009–14. [PubMed: 21069489]

50. Pazhenkottil AP, Buechel RR, Herzog BA, Nkoulou RN, Valenta I, Fehlmann U, et al. Ultrafast assessment of left ventricular dyssynchrony from nuclear myocardial perfusion imaging on a new high-speed gamma camera. *Eur J Nucl Med Mol Imaging*. 2010; 37:2086–92. [PubMed: 20556604]
51. Schillaci O, Danieli R. Dedicated cardiac cameras: a new option for nuclear myocardial perfusion imaging. *Eur J Nucl Med Mol Imaging*. 2010; 37:1706–9. [PubMed: 20593283]
52. Fiechter M, Ghadri JR, Kuest SM, Pazhenkottil AP, Wolfrum M, Nkoulou RN, et al. Nuclear myocardial perfusion imaging with a novel cadmium-zinc-telluride detector SPECT/CT device: first validation versus invasive coronary angiography. *Eur J Nucl Med Mol Imaging*. 2011; 38:2025–30. [PubMed: 21761267]
53. Srivastava PC, Knapp FF Jr. [(E)-1-[¹²³I]Iodo-1-penten-5-yl]triphenylphosphonium iodide: convenient preparation of a potentially useful myocardial perfusion agent. *J Med Chem*. 1984; 27:978–81. [PubMed: 6747996]
54. Srivastava PC, Hay HG, Knapp FF Jr. Effects of alkyl and aryl substitution on the myocardial specificity of radioiodinated phosphonium, arsonium, and ammonium cations. *J Med Chem*. 1985; 28:901–4. [PubMed: 4009613]
55. Krause BJ, Szabo Z, Becker LC, Dannals RF, Scheffel U, Seki C, et al. Myocardial perfusion with [¹¹C]methyl triphenyl phosphonium: measurements of the extraction fraction and myocardial uptake. *J Nucl Biol Med*. 1994; 38:521–6. [PubMed: 7865551]
56. Madar I, Ravert HT, Du Y, Hilton J, Volokh L, Dannals RF, et al. Characterization of uptake of the new PET imaging compound ¹⁸F-fluorobenzyltriphenyl phosphonium in dog myocardium. *J Nucl Med*. 2006; 47:1359–66. [PubMed: 16883017]
57. Kim DY, Kim HS, Le UN, Jiang SN, Kim HJ, Lee KC, et al. Evaluation of a mitochondrial voltage sensor, (¹⁸F-fluoropentyl)triphenylphosphonium cation, in a rat myocardial infarction model. *J Nucl Med*. 2012; 53:1779–85. [PubMed: 23038748]
58. Kim DY, Kim HJ, Yu KH, Min JJ. Synthesis of [¹⁸F]-labeled (6-fluorohexyl)triphenylphosphonium cation as a potential agent for myocardial imaging using positron emission tomography. *Bioconjugate Chem*. 2012; 23:431–437.
59. Kim DY, Kim HJ, Yu KH, Min JJ. Synthesis of [¹⁸F]-labeled (2-(2-fluoroethoxy)ethyl)triphenylphosphonium cation as a potential agent for myocardial imaging using positron emission tomography. *Bioorg Med Chem Lett*. 2012; 22:319–22. [PubMed: 22133630]
60. Madar I, Ravert H, Nelkin B, Abro M, Pomper M, Dannals R, et al. Characterization of membrane potential-dependent uptake of the novel PET tracer ¹⁸F-fluorobenzyl triphenylphosphonium cation. *Eur J Nucl Med Mol Imaging*. 2007; 34:2057–65. [PubMed: 17786439]
61. Kim DY, Yu KH, Bom HS, Min JJ. Synthesis of (4-[¹⁸F]fluorophenyl)triphenylphosphonium as a mitochondrial voltage sensor for PET. *Nucl Med Mol Imaging*. 2007; 41:561–5.
62. Madar I, Ravert H, Dipaula A, Du Y, Dannals RF, Becker L. Assessment of severity of coronary artery stenosis in a canine model using the PET agent ¹⁸F-fluorobenzyl triphenyl phosphonium: comparison with ^{99m}Tc-tetrofosmin. *J Nucl Med*. 2007; 48:1021–30. [PubMed: 17504876]
63. Higuchi T, Fukushima K, Rischpler C, Isoda T, Javadi MS, Ravert H, et al. Stable delineation of the ischemic area by the PET perfusion tracer ¹⁸F-fluorobenzyl triphenyl phosphonium after transient coronary occlusion. *J Nucl Med*. 2011; 52:965–9. [PubMed: 21571789]
64. Ross MF, Kelso GF, Blaikie FH, James AM, Cocheme HM, Filipovska A, et al. Lipophilic triphenylphosphonium cations as tools in mitochondrial bioenergetics and free radical biology. *Biochemistry (Mosc)*. 2005; 70:222–30. [PubMed: 15807662]
65. Pasqualini R, Duatti A. Synthesis and characterization of the new neutral myocardial imaging agent [^{99m}TcN(noet)₂](noet = N-ethyl-N-ethoxydithiocarbamate). *J Chem Soc Chem Commun*. 1992; 18:1354–5.
66. Pasqualini R, Duatti A, Bellande E, Comazzi V, Brucato V, Hoffschir D, et al. Bis(dithiocarbamate) nitrido technetium-99m radiopharmaceuticals: a class of neutral myocardial imaging agents. *J Nucl Med*. 1994; 35:334–41. [PubMed: 8295007]
67. Riou LM, Ghezzi C, Mouton O, Mathieu JP, Pasqualini R, Comet M, et al. Cellular uptake mechanisms of ^{99m}TcN-NOET in cardiomyocytes from newborn rats: calcium channel interaction. *Circulation*. 1998; 98:2591–7. [PubMed: 9843468]

68. Riou LM, Ghezzi C, Vanzetto G, Broisat A, Mathieu JP, Bontronl R, et al. Verapamil does not inhibit ^{99m}Tc -NOET uptake in situ in normal or ischemic canine myocardium. *J Nucl Med.* 2003; 44:981–7. [PubMed: 12791829]

Chart I.

Schematic Illustration for Radiosynthesis of $^{99\text{m}}\text{Tc}$ -Teboroxime, $^{99\text{m}}\text{Tc}$ -Teboroxime(F), $^{99\text{m}}\text{Tc}$ -Teboroxime(N_3) and $^{99\text{m}}\text{Tc}$ -Teboroxime(SCN).

Initial loss of water molecule to form a cationic intermediate $[^{99m}\text{Tc}(\text{CDO})_2(\text{CDOH})_2\text{BMe}]^+$, in which $^{99m}\text{Tc}(\text{III})$ is 6-coordinated, followed by addition of the $\text{F}^-/\text{SCN}^-/\text{N}_3^-$ anion to form neutral complexes $[^{99m}\text{Tc}(\text{L})(\text{CDO})_2(\text{CDOH})_2\text{BMe}]$ ($\text{L} = \text{F}, \text{SCN}$ and N_3).

Initial attack by the $\text{F}^-/\text{SCN}^-/\text{N}_3^-$ anion to form an 8-coordinate intermediate, followed by loss of water molecule to form complexes $[^{99m}\text{Tc}(\text{L})(\text{CDO})_2(\text{CDOH})_2\text{BMe}]$ ($\text{L} = \text{F}, \text{SCN}$ and N_3).

Base attack of the dioxime backbone with removal of a proton and rapid loss of water molecule to form the 6-coordinated neutral intermediate $[^{99m}\text{Tc}(\text{CDO})(\text{CDO}-\text{H}_2\text{O})(\text{CDOH})\text{BMe}]$. The final step involves attack by the $\text{F}^-/\text{SCN}^-/\text{N}_3^-$ anion and re-protonation of the dioxime backbone.

Chart II.

Three Possible Mechanisms for Ligand Exchange Reaction.

^{99m}Tc -Teboroxime: L = Cl

^{99m}Tc -Teboroxime(F): L = F

^{99m}Tc -Teboroxime(SCN): L = SCN

^{99m}Tc -Teboroxime(N_3): L = N_3

Figure 1.

Structures of ^{99m}Tc (III) radiotracers [$^{99m}\text{Tc}(\text{L})(\text{CDO})(\text{CDOH})_2\text{BMe}$] (^{99m}Tc -Teboroxime: L = Cl; ^{99m}Tc -Teboroxime(F): L = F; ^{99m}Tc -Teboroxime(SCN): L = SCN; and ^{99m}Tc -Teboroxime(N_3): L = N_3). ^{99m}Tc -Teboroxime was the first FDA-approved radiotracer for MPI; but it was later withdrawn from the market due to its fast washout from heart. ^{99m}Tc -Teboroxime(F), ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N_3) are new radiotracers evaluated in this study.

Figure 2. Radio-HPLC chromatograms of ^{99m}Tc -Teboroxime (RCP >95%), ^{99m}Tc -Teboroxime(F) (RCP = 85 – 90%), ^{99m}Tc -Teboroxime(SCN) (RCP = 90 – 93% for the sum of two radiometric peaks at 16.4 and 18.4 min) and ^{99m}Tc -Teboroxime(N₃) (RCP >95%).

Figure 3.

Planar images of the SD rats administered with ^{99m}Tc -Teboroxime, ^{99m}Tc -Teboroxime(F), ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N_3). Animals were anesthetized with intramuscular injection of a mixture of ketamine (80 mg/kg) and xylazine (19 mg/kg). Each animal was injected with 1.0 – 1.5 mCi of ^{99m}Tc radiotracer. The 1-min static images were acquired over the first 5 min post-injection, followed by the 2-min static images at 6 – 30, 40, 50 and 60 min p.i. Arrows indicate the presence of radioactivity accumulation in the heart. White circles indicate the radioactivity accumulation in the blood vessels. ^{99m}Tc -Teboroxime(F) had a very fast washout from the heart. ^{99m}Tc -Teboroxime and ^{99m}Tc -Teboroxime(N_3) showed significant radioactivity accumulation in blood vessels.

Figure 4.

Image quantification data to compare the heart retention times of ^{99m}Tc -Teboroxime, ^{99m}Tc -Teboroxime(F), ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N_3). The experimental data were expressed as the total heart uptake (%ID). The heart retention curve was best fitted to a bi-exponential function using the individual animal time/activity data.

Figure 5.

Image quantification data to compare the liver clearance kinetics of ^{99m}Tc -Teboroxime, ^{99m}Tc -Teboroxime(F), ^{99m}Tc -Teboroxime(SCN) and ^{99m}Tc -Teboroxime(N_3). These data were expressed as % of the initial liver uptake at 0 – 1 min. The liver clearance curve was best fitted to a bi-exponential function using the individual animal time/radioactivity data. It is impossible to quantify the “absolute liver radioactivity level” because there was no clear separation between liver and surrounding organs.

Figure 6. Direct comparison of the 2-min heart uptake (%ID/g) for ^{99m}Tc -Sestamibi, ^{99m}TcN -MPO, ^{99m}Tc -Teboroximine and ^{99m}Tc -Teboroxime(N_3).

Figure 7.

A: Coronal views of SPECT images of the SD rats administered with 80 – 90 MBq of ^{99m}Tc -Teboroxime (upper panel) or ^{99m}Tc -Teboroxime(N_3) (lower panel). Anesthesia was induced using an air flow rate of 350 mL/min and ~3.0% isoflurane. SPECT images were obtained over the first 5 min with camera being focused in the heart region. Arrows indicate the presence of the liver radioactivity. Despite intense liver uptake, high quality SPECT images could be acquired. **B:** The 3D views of SPECT/CT images of the SD rats administered with 80 – 90 MBq of ^{99m}Tc -Teboroxime or ^{99m}Tc -Teboroxime(N_3) to illustrate the radioactivity accumulation in the heart region. The radioactivity in the blood vessels (above the heart) was clearly seen in the SPECT/CT image of the SD rat administered with ^{99m}Tc -Teboroxime(N_3).

Table 1

Selected biodistribution data for ^{99m}Tc -Teboroxime in SD rats (n = 4).

Organ	2 min	15 min	30 min	60 min
Blood	0.47 ± 0.05	0.23 ± 0.05	0.31 ± 0.09	0.22 ± 0.05
Brain	0.13 ± 0.02	0.05 ± 0.01	0.08 ± 0.04	0.05 ± 0.01
Heart	3.00 ± 0.37	1.25 ± 0.09	0.86 ± 0.17	0.58 ± 0.04
Intestines	1.04 ± 0.20	1.23 ± 0.35	1.84 ± 0.32	0.81 ± 0.09
Kidneys	3.21 ± 0.38	2.18 ± 0.80	1.97 ± 0.17	0.68 ± 0.45
Liver	2.67 ± 0.47	2.40 ± 0.67	2.22 ± 0.17	0.95 ± 0.16
Lungs	2.90 ± 0.35	1.64 ± 0.28	1.41 ± 0.25	0.94 ± 0.25
Muscle	0.45 ± 0.10	0.33 ± 0.01	0.27 ± 0.07	0.21 ± 0.05
Spleen	2.33 ± 0.58	1.38 ± 0.11	1.14 ± 0.04	0.42 ± 0.10
Heart/Blood	6.42 ± 1.26	5.55 ± 0.95	2.93 ± 0.90	2.88 ± 1.04
Heart/Lung	1.07 ± 0.12	0.78 ± 0.12	0.61 ± 0.04	0.66 ± 0.21
Heart/Liver	1.18 ± 0.21	0.56 ± 0.16	0.39 ± 0.09	0.62 ± 0.12
Heart/Muscle	6.12 ± 2.03	3.82 ± 0.19	3.57 ± 1.49	2.90 ± 0.67

Table 2

Selected biodistribution data for ^{99m}Tc -Teboroxime(N_3) in SD rats (n = 6).

Organ	2 min	15 min	30 min	60 min
Blood	0.30 ± 0.05	0.14 ± 0.01	0.13 ± 0.00	0.07 ± 0.02
Brain	0.16 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.06 ± 0.01
Heart	2.66 ± 0.01	0.90 ± 0.10	0.51 ± 0.02	0.29 ± 0.02
Intestines	0.73 ± 0.05	0.76 ± 0.02	0.92 ± 0.06	0.76 ± 0.23
Kidneys	2.49 ± 0.13	0.91 ± 0.08	0.73 ± 0.10	0.41 ± 0.05
Liver	3.68 ± 0.09	2.30 ± 0.08	1.57 ± 0.08	0.70 ± 0.12
Lungs	1.77 ± 0.21	0.57 ± 0.04	0.45 ± 0.01	0.25 ± 0.04
Muscle	0.19 ± 0.06	0.23 ± 0.00	0.19 ± 0.04	0.16 ± 0.01
Spleen	1.35 ± 0.04	0.45 ± 0.01	0.38 ± 0.07	0.21 ± 0.01
Vessels	1.29 ± 0.51	1.20 ± 0.67	1.10 ± 0.42	0.82 ± 0.34
Heart/Blood	9.02 ± 0.52	6.41 ± 1.33	3.99 ± 0.31	4.14 ± 0.57
Heart/Lung	1.53 ± 0.18	1.60 ± 0.29	1.12 ± 0.06	1.16 ± 0.09
Heart/Liver	0.72 ± 0.02	0.39 ± 0.06	0.32 ± 0.00	0.41 ± 0.04
Heart/Muscle	15.56 ± 5.15	3.85 ± 0.44	2.79 ± 0.71	1.78 ± 0.06