Published in final edited form as:

J Nucl Cardiol. 2010 October ; 17(5): 858–867. doi:10.1007/s12350-010-9262-y.

Kinetic characterization of a novel cationic $^{99m}\text{Tc}(\text{I})$ -tricarbonyl complex, ^{99m}Tc -15C5-PNP, for myocardial perfusion imaging

Zhonglin Liu, MD¹, Liying Chen, PhD¹, Shuang Liu, PhD², Christy Barber, BS¹, Gail D. Stevenson, DVM¹, Lars R. Furenlid, PhD¹, Harrison H. Barrett, PhD¹, and James M. Woolfenden, MD¹

¹ Department of Radiology, University of Arizona, Tucson, Arizona

² School of Health Sciences, Purdue University, West Lafayette, Indiana

Abstract

Background—Intense liver uptake of ^{99m}Tc -sestamibi (MIBI) often interferes with visualization of myocardial perfusion in the inferior wall of the left ventricle. To develop improved myocardial perfusion agents, crown ether-containing dithiocarbamates and bisphosphines have been introduced in recent years. This study was designed to investigate the myocardial imaging properties and *in vivo* kinetics of a cationic $^{99m}\text{Tc}(\text{I})$ -tricarbonyl complex, ^{99m}Tc -15C5-PNP, in comparison with MIBI.

Methods—Dynamic cardiac images were acquired for 60 minutes after intravenous tracer injection using a small-animal SPECT system in healthy control rats and rats with myocardial infarcts. Myocardial and liver time-activity curves were generated for radiopharmaceutical kinetic analysis.

Results—Good visualization of the left ventricular wall and perfusion defects could be achieved 20 min after ^{99m}Tc -15C5-PNP administration. ^{99m}Tc -15C5-PNP images in all hearts with infarcts showed perfusion defects, which were comparable to MIBI images. The kinetic curves plotted from 1 to 60 min demonstrated that ^{99m}Tc -15C5-PNP has a shorter washout half-life (6.4 ± 3.2 min vs. 124 ± 30.5 min, $P < 0.01$) in the liver, lower residual liver activity ($14.5 \pm 10.2\%$ vs. $36.5 \pm 28.9\%$, $P < 0.01$), and higher heart/liver ratio than MIBI.

Conclusions— ^{99m}Tc -15C5-PNP has potential for rapid myocardial perfusion imaging with low liver uptake

Keywords

$^{99m}\text{Tc}(\text{I})$ -tricarbonyl complex; Ischemia; Heart; Rat; SPECT

INTRODUCTION

The technetium-99m-labeled cationic myocardial imaging agents ^{99m}Tc -sestamibi (MIBI) and ^{99m}Tc -tetrofosmin are FDA-approved perfusion radiotracers for functional myocardial assessment.^{1–3} These monocationic agents offer physical advantages over ^{201}Tl and share the properties of lipophilicity, small molecular size and monocationic charge, but they suffer from high liver uptake and increased bowel activity because the hepatobiliary system is the major metabolic pathway for clearance. The problems of high liver uptake and increased bowel activity interfere with visual and quantitative interpretation of the inferior wall of the left

ventricle, particularly in rest images. Despite many efforts to reduce liver and bowel interference by stimulating hepatobiliary clearance of the bile,^{4–8} there is no conclusive evidence that these treatments work for every patient. Photon scattering from high liver activity remains a significant challenge for diagnosis of heart disease. In addition, neither ^{99m}Tc-sestamibi nor ^{99m}Tc-tetrofosmin is an ideal perfusion agent due to their inability to track high myocardial blood flow.⁹ Thus, there is an unmet medical need for a radiotracer with high heart uptake and better heart/liver ratio than that of ^{99m}Tc-sestamibi/^{99m}Tc-tetrofosmin.

Over the past decade, investigators have been working on developing novel improved myocardial agents having imaging characteristics much closer to those of an ideal perfusion indicator, including high myocardial tracer extraction with stable myocardial retention, linear tracking of myocardial blood flow over a wide range, and minimal accumulation in the liver or a rapid decrease in liver activity.^{10–12} As a result of these efforts, new cationic ^{99m}Tc-nitrido complexes and ^{99m}Tc(I)-tricarbonyl complexes have been introduced in recent years, in which crown ether functional groups are used to improve liver clearance.^{12–16} The unique chemical feature of these compounds lies in the fact that their molecular structure is composed of two different bidentate ligands bound to the same Tc≡N group, thereby giving rise to asymmetrical complexes carrying a monopositive charge.¹⁰ Among these crown ether-containing agents, a ^{99m}Tc-nitrido complex, ^{99m}Tc-[bis(dimethoxypropylphosphinoethyl)-ethoxyethylamine (PNP5)]-[bis(*N*-ethoxyethyl)-dithiocarbamate (DBODC)] nitride or ^{99m}Tc-N-DBODC5, has been studied in various animal models and is currently under clinical investigation as a new myocardial perfusion imaging agent.^{12, 14, 17} Another ^{99m}Tc nitrido complex, ^{99m}Tc-N-MPO ([^{99m}Tc N(MPO)(PNP5)]), also shows promising results in rat models.^{18, 19}

[^{99m}Tc(CO)₃(15C5-PNP)]⁺, simplified as ^{99m}Tc-15C5-PNP, is a crown ether-containing ^{99m}Tc(I)-tricarbonyl complex, where 15C5-PNP equals N-[15-crown-5)-2-yl]-N,N-bis[2-(bis(3-ethoxypropyl)phosphino)ethyl]amine. The strong bonding of bisphosphine ligands to the [^{99m}Tc(CO)₃]⁺ core gives this tricarbonyl complex high solution stability and rapid clearance from blood and nontarget organs.¹⁵ The results from biodistribution measurements in rats and cardiac imaging studies in dogs indicate that this compound holds promise as a new radiotracer for rapid myocardial perfusion imaging in view of its high initial heart uptake and rapid clearance from the liver.^{13, 15} However, the *in vivo* radiopharmaceutical kinetics of ^{99m}Tc-15C5-PNP have not been well studied, especially regarding its liver clearance in comparison with ^{99m}Tc-sestamibi. The present study was designed to investigate the imaging properties and *in vivo* kinetics of ^{99m}Tc-15C5-PNP in comparison with ^{99m}Tc-sestamibi in a rat model with myocardial infarction using a small-animal SPECT system, FastSPECT II.

MATERIALS AND METHODS

Radiopharmaceutical preparation

The bisphosphine ligand 15C5-PNP was developed at Purdue University, West Lafayette, Indiana. IsoLink vials were obtained as a gift from Mallinckrodt, Inc. (St. Louis, Missouri). The cationic ^{99m}Tc(I)-tricarbonyl complex was prepared (Scheme II) using a radiolabeling procedure similar to previous publications.²⁰ ^{99m}TcO₄ (60 mCi) in 1.0 mL saline was added to the IsoLink vial. The reaction mixture was heated at 100°C for 20 minutes to form the ^{99m}Tc(CO)₃(H₂O)₃⁺ intermediate. After cooling to room temperature, the solution was neutralized with 0.2 mL of a 1:2 mixture of 1 M phosphate buffer (pH 7.4) and 1 M HCl. The solution (0.2 mL) containing ^{99m}Tc(CO)₃(H₂O)₃⁺ was added into a 10 mL vial containing 0.05 mL of a 10^{−2} M solution of the bisphosphine ligand in 0.25 mL physiological phosphate buffer (PBS). The mixture was incubated at room temperature for 30 minutes. The radiochemical purity was determined by instant thin-layer chromatography (ITLC-SG; Gelman Sciences) using 2 solvent systems as the mobile phase: saline and NH₃.H₂O/alcohol/H₂O (1:2:5). The

radiochemical purity (RCP) of ^{99m}Tc -15C5-PNP exceeded 95% for all experimental injections. ^{99m}Tc -15C5-PNP was used within 6 hours after labeling.

^{99m}Tc -sestamibi was prepared with a Cardiolite® kit (Lantheus Medical Imaging) provided by Cardinal Health. The RCP was greater than 95%.

Animal models and experimental groups

The rat model of myocardial infarction was developed using the technique described previously.²¹ Male Sprague-Dawley rats (250–300 g) were initially anesthetized with sodium pentobarbital (50 mg/kg) injected intraperitoneally. After intubation, respiration was maintained using a volume-controlled Inspira Advanced Safety Ventilator (Harvard Apparatus, Holliston, Massachusetts) with a mixture of oxygen and room air. The chest was opened at the fourth or fifth intercostal space through a left intercostal thoracotomy incision. The left coronary artery (LCA) was permanently occluded using a 6.0 Prolene suture at about 1 mm below the left atrial appendage. The onset of acute ischemia was confirmed by the pale appearance in the area-at-risk region immediately upon occlusion as well as changes in ECG, including elevation of the ST segment and a significant increase in the QRS complex amplitude and width. The chest wall was closed in sutured layers, and ventilation was maintained until the end of the experiment.

^{99m}Tc -15C5-PNP and sestamibi were studied in both healthy control rats and the rats with myocardial infarction. Immediately after intravenous injection, dynamic ^{99m}Tc -15C5-PNP images were acquired for 60 minutes in 5 healthy rats (Group 1, ^{99m}Tc -15C5-PNP/control) and 8 rats with myocardial ischemia (Group 2, ^{99m}Tc -15C5-PNP/ischemia). Using the same imaging protocol, ^{99m}Tc -sestamibi (MIBI) images were collected in 6 control rats (Group 3, MIBI/control) and 7 rats with myocardial ischemia (Group 4, MIBI/ischemia).

Dynamic high-resolution SPECT imaging

The rats were imaged in list-mode acquisition by FastSPECT II, a stationary small animal SPECT imager built at the Center for Gamma-Ray Imaging (CGRI) of the University of Arizona.²² The system consists of 16 modular cameras and a cylindrical aperture with 16 1-mm-diameter pinholes. FastSPECT II provides dynamic imaging capabilities without rotation of either animals or detectors. The spatial resolution is about 1.0 mm, and the system sensitivity in this study is 0.27 cps/kBq.

The anesthetized rat was placed inside the aperture using a translation stage. The animal was positioned so that the field of view of the SPECT imager could cover the entire chest and upper abdomen. At 90 minutes of LCA ligation, ^{99m}Tc -15C5-PNP or ^{99m}Tc -sestamibi (111–148 MBq, 0.5 mL) was injected intravenously via a pre-installed jugular vein catheter using a Harvard PHD2000 syringe pump (Harvard Apparatus, Holliston, Massachusetts), followed by a 0.1-mL saline flush. Immediately after injection, dynamic images in list-mode acquisition were acquired every minute for 10 minutes, followed by 5-minute acquisitions at 15, 30, 45, and 60 minutes post-injection. A total of 16 projections were obtained, one from each camera, to generate the data set for tomographic reconstruction.

Image processing

Tomographic reconstructions of FastSPECT II data were processed using 25 iterations of the OS-EM algorithm with 4 subsets, 4 projections per subset. The reconstructed data were computed to provide 3D images in a $41 \times 41 \times 41$ voxel format. The oblique re-orientation of transaxial data was performed by computerized procedures to generate tomographic short-axis (transverse), coronal, and sagittal slices with 1-voxel thickness (1.0 mm).

Using AMIDE 0.9.1 software, 3D region-of-interest (ROI) analysis was applied to generate myocardial time-activity curves (TACs). The ROIs were established over normal myocardial zones supplied by the right coronary artery (RCA) and over perfusion defect areas on all transverse slices from the base to the apex in the 60-minute images. The 60-minute ROIs were applied to all of the dynamic images for determining average counts per pixel at 1–10, 15, 30, 45, and 60 minutes post-injection. After correction for acquisition time and decay, myocardial TACs over the normal zones and infarct areas were plotted by normalizing radioactive counts at each time point to peak uptake counts. TACs were also normalized to counts at 10 minutes post-injection to minimize contributions from blood-pool activity. Based on the TACs, the average fractional washout and retention of the myocardium at each time-point were calculated.

Similarly, the ROI analysis was applied on all coronal slices of the liver to obtain the average liver TACs. TableCurve 2D[®] software (Systat Software Inc., Chicago, IL) was applied to fit the TACs using a set of kinetic functions with or without equilibrium. Because the liver TACs of ^{99m}Tc-sestamibi did not reach a constant level within 60 minutes, we used the First Order Intermediate Equation (Non-linear Equation 8134) with no equilibrium to fit the kinetic data and generate the best fitted liver curves. The kinetic data of ^{99m}Tc-15C5-PNP radioactivity were fitted in the First Order Intermediate with Equilibrium (Non-linear Equation 8130). Subsequently, we chose to include the equilibrium terms for fitting ^{99m}Tc-sestamibi TACs to make the kinetic data of ^{99m}Tc-sestamibi comparable to that of ^{99m}Tc-15C5-PNP. We described the liver-imaging kinetics by multiple parameters calculated from the equation set, including the Peak Time (T_p), Peak Activity (Peak), Washout Half-Life (washout time to 50% of peak activity, T_{1/2}), and Equilibrium (Residual Activity Retention) of each individual animal. To avoid the variation of injected dose, the peak value was generated using normalized individual activity per pixel, which was the percentage of its initial radioactivity, and not expressed as counts per pixel per minute. All the parameters were then averaged over all the healthy rats and the rats with infarcts for both ^{99m}Tc-sestamibi and ^{99m}Tc-15C5-PNP.

Histological and autoradiograph analysis

Postmortem histological and autoradiograph analyses were performed after FastSPECT II imaging to confirm the distribution of ^{99m}Tc-15C5-PNP in cardiac tissues. Each rat was sacrificed by intraperitoneal injection of an overdose of sodium pentobarbital (200 mg/kg). Prior to sacrifice, Evans blue dye (10%) in 1.0 PBS buffer was injected through the femoral vein to stain the nonischemic portion of the heart and determine the myocardial ischemic area-at-risk (IAR), which was identified as that region lacking blue staining in the rats with myocardial ischemia. The left ventricle was sectioned into 4–5 transverse slices in a plane parallel to the atrioventricular groove. Both sides of each tissue slice were photographed immediately using a digital camera. Triphenyltetrazolium chloride (TTC) staining was used to identify the area with infarct. The viable myocardium stained by TTC exhibited a dark red color. Both sides of each TTC-stained tissue slice were photographed again with the digital camera.

The spatial distribution of radioactivity was examined using autoradiography. The tissue slices were covered with 1 layer of plastic wrap and exposed to the FujiFilm phosphor imaging plates for 5–15 minutes. A FujiFilm BAS5000 Bio-Imaging Analysis System (Stamford, Connecticut) was used to scan the plates for digital autoradiograph collection. The radioactivity of the myocardium was quantitatively analyzed with the software of SigmaScan Pro 5.0 (SPSS Science, Chicago, IL) in trace-measurement mode. The average intensity of autoradiograph images at gray-scale display in normal and ischemic myocardium was determined and the ratio of ischemic zone/normal zone was calculated.

Biodistribution Measurements

The animals were sacrificed and tissue samples were collected for biodistribution measurements. The blood, heart, lung, muscle, liver, spleen, intestine, stomach, and kidneys were weighed and measured using a CRC-15W radioisotope dose calibrator (Capintec, Ramsey, NJ). After radioactive-decay correction, the radioactivity in the tissue samples was expressed as percentage of injected dose per gram of tissue (%ID/g).

Data analysis

All quantitative results were expressed as mean $\pm$ S.E.M. Comparisons between two variables were performed with one-way analysis of variance. Probability values less than 0.05 were considered significant.

Ethics

The animal experiments were performed in accordance with the Principles of Laboratory Animal Care from the National Institutes of Health (NIH Publication 85–23, revised 1985) and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Arizona.

RESULTS

Radioactive distribution in the heart

The *in vivo* kinetic profiles of ^{99m}Tc -15C5-PNP in the healthy rat hearts and infarcted hearts were obtained by dynamic tomographic imaging. The blood-pool signals were evident after tracer injection on the 1-minute images of ^{99m}Tc -15C5-PNP. Two minutes post-injection, the spatial distribution of radioactivity in the viable myocardium was noted and remained prominent over the 60-minute post-injection period. As shown in Figure 1, the wall of the left ventricle was well visualized by ^{99m}Tc -15C5-PNP as compared to ^{99m}Tc -sestamibi. In the ischemic hearts, the areas of infarcted myocardium exhibited distinct focal radioactive defects in the lateral and anterior wall of the left ventricle on ^{99m}Tc -15C5-PNP and ^{99m}Tc -sestamibi images (see Figure 2). Typically, the infarct lesions were clearly detectable on ^{99m}Tc -15C5-PNP images 15 minutes after intravenous tracer administration. ^{99m}Tc -15C5-PNP cardiac images were comparable to ^{99m}Tc -sestamibi images in demonstrating myocardial perfusion and defects.

The hearts with myocardial infarction were evaluated post-mortem by TTC staining and autoradiography. Representative examples of Evans blue dye and TTC staining, and autoradiograph images of ischemic hearts in ^{99m}Tc -15C5-PNP/ischemia and ^{99m}Tc -sestamibi/ischemia groups are shown in Figure 3. The radioactive defects of ^{99m}Tc -15C5-PNP and ^{99m}Tc -sestamibi on autoradiograph images were consistent with the unstained areas on Evans blue staining and TTC staining. ^{99m}Tc -15C5-PNP was comparable to ^{99m}Tc -sestamibi in illustrating viable and infarcted myocardium. The radioactive ratios of non-ischemic myocardium to ischemic myocardium were 2.27 ± 0.24 for ^{99m}Tc -15C5-PNP and 2.60 ± 0.49 for ^{99m}Tc -sestamibi ($P > 0.05$). Overall, autoradiograph imaging showed no significant difference between ^{99m}Tc -15C5-PNP and ^{99m}Tc -sestamibi distribution in non-ischemic and ischemic myocardium.

Radioactive distribution in the liver

Immediately after intravenous injection, accumulation of ^{99m}Tc -15C5-PNP and ^{99m}Tc -sestamibi was observed in the liver. ^{99m}Tc -sestamibi radioactivity in the liver remained high and exhibited no significant washout within 60 minutes after injection. As shown in Figure 4, the intense liver uptake made it difficult to interpret the heart activity in the inferior

wall of the left ventricle. In contrast, the radioactive level of ^{99m}Tc -15C5-PNP in the liver quickly declined so that the inferior wall of the left ventricle could be well distinguished by 15–20 minutes post-injection.

Kinetic analysis of dynamic image

Myocardial TACs were generated using computerized ROI analysis with decay and acquisition-time correction. Tracer kinetic profiles of ^{99m}Tc -15C5-PNP and ^{99m}Tc -sestamibi in the heart are shown in Figure 5. In the healthy control hearts, there was no significant difference among the segments of the left ventricle in terms of radioactivity. A significant difference was observed at each time point from 5 minutes to 60 minutes between the LCA ischemic area and normal zone on both ^{99m}Tc -15C5-PNP and ^{99m}Tc -sestamibi images. At 60 minutes after injection, the average radioactive ratio of non-ischemic zone to ischemic area in ischemic hearts was 11.4 ± 3.7 for ^{99m}Tc -15C5-PNP and 31.0 ± 1.9 for ^{99m}Tc -sestamibi ($P < 0.05$). In contrast, the ratio of RCA area to LCA zone in the control hearts was 0.93 ± 0.09 for ^{99m}Tc -15C5-PNP and 1.10 ± 0.09 for ^{99m}Tc -sestamibi ($P > 0.05$). Relative to ^{99m}Tc -sestamibi, ^{99m}Tc -15C5-PNP exhibited a significantly greater washout from the normal myocardium. Fractional washout of the tracers is summarized in Table 1. The fractional washout of ^{99m}Tc -15C5-PNP was 2.3–3.2 times higher than that of ^{99m}Tc -sestamibi from normal myocardium ($P < 0.05$). Because of the permanent coronary occlusion, radiotracer activity was close to zero in the ischemic area at the end imaging session. The apparent washout of activity between 1 and 10 minutes in ischemic myocardium is consistent with clearance of blood-pool activity. After 10 minutes post-injection, no radioactivity washout was observed from ischemic myocardium with either ^{99m}Tc -15C5-PNP or ^{99m}Tc -sestamibi. The fractional washout from 10 minutes to 60 minutes post-injection in the ischemic area was zero.

As shown in Figure 6, ^{99m}Tc -15C5-PNP reached its maximum level approximately half of the time as ^{99m}Tc -sestamibi and took only 30 minutes to get to its residue level, which is more than 2 times as low as that of ^{99m}Tc -sestamibi. The liver curve of ^{99m}Tc -sestamibi shown in Figure 6 was plotted using the First Order Intermediate Equation with no equilibrium. Typically, the radioactivity of ^{99m}Tc -sestamibi in the liver started to show a slow washout at 30 minutes post-injection reached a statistically lower level until 60 minutes. The kinetic results of ^{99m}Tc -sestamibi and ^{99m}Tc -15C5-PNP in the liver are summarized in Table 2. The time-to-peak of ^{99m}Tc -15C5-PNP was shorter than that of ^{99m}Tc -sestamibi, although there was no significant difference in peak uptake levels. During the 1-hour acquisition period, ^{99m}Tc -15C5-PNP washed out significantly faster than ^{99m}Tc -sestamibi with a washout half life about 20 times shorter than that of ^{99m}Tc -sestamibi. As a result, ^{99m}Tc -15C5-PNP had significantly lower residual activity in the liver than ^{99m}Tc -sestamibi ($P < 0.05$).

Biodistribution measurements

The tissue activity distributions of ^{99m}Tc -sestamibi and ^{99m}Tc -15C5-PNP at 60 minutes post-injection are summarized in Table 3. The organs with greatest ^{99m}Tc -15C5-PNP activity were the intestine and kidneys. The heart activity of ^{99m}Tc -15C5-PNP was significantly lower than that of ^{99m}Tc -sestamibi. However, ^{99m}Tc -sestamibi demonstrated significantly higher liver uptake than ^{99m}Tc -15C5-PNP. As a result, the heart-liver ratio of ^{99m}Tc -15C5-PNP was greater than that of ^{99m}Tc -sestamibi (3.11 ± 0.38 vs. 1.71 ± 0.14 in IR, 3.31 ± 0.32 vs. 1.77 ± 0.21 in Control, $P < 0.01$, respectively).

DISCUSSION

Various ether-containing ligands and chelators have been used for the preparation of cationic ^{99m}Tc complexes in attempts to improve myocardial target/background (T/B) ratios. Approaches that introduce crown ether groups into cationic ^{99m}Tc -nitrido and tricarbonyl

complexes include the use of crown ether-containing dithiocarbamates and bisphosphine.^{13, 18, 23} The crown ethers containing dithiocarbamates form highly stable cationic ^{99m}Tc -nitrido complexes and decrease the lipophilicity of the complexes without changing the overall charge. Lipophilicity is an important factor influencing the biodistribution characteristics of ^{99m}Tc -labeled cationic radiotracers. Cationic ^{99m}Tc -labeled radiotracers with log P (partition coefficient) >1.5 often have high protein binding and slow liver clearance, while more hydrophilic cationic radiotracers with log $P < 0$ tend to show a fast washout from the myocardium.²³ Cationic ^{99m}Tc radiotracers must have a log P value of 0.5–1.2 in order to achieve high myocardial uptake with fast liver clearance at the same time.

^{99m}Tc -N-DBODC5 exhibits high myocardial uptake like ^{99m}Tc -sestamibi and ^{99m}Tc -tetrafosmin, but it has faster and greater liver clearance than these two agents in rat and canine models,^{12, 14, 17, 24} suggesting that it may allow more accurate assessment of myocardial perfusion with less photon scatter from the liver. ^{99m}Tc -N-MPO is another new monocationic agent that has molecular charge similar to ^{99m}Tc -sestamibi and shows rapid clearance from the liver and lungs, resulting in high heart/liver and heart/lung ratios.^{18, 19} The kinetics of ^{99m}Tc -N-MPO, ^{99m}Tc -N-DBODC5, and ^{99m}Tc -sestamibi differ significantly in rats, possibly because of differences in molecular shape. The heart/liver ratio of ^{99m}Tc -N-MPO at 30 minutes after injection is about 4 times higher than that of ^{99m}Tc -sestamibi and 2 times higher than that of ^{99m}Tc -N-DBODC5¹⁸, suggesting that ^{99m}Tc -N-MPO is an attractive candidate for myocardial perfusion imaging.

Previous studies have shown that ^{99m}Tc -15C5-PNP has a log P value of 0.86 ± 0.05 , and its biodistribution characteristics are similar to those of ^{99m}Tc -N-DBODC5 in terms of heart uptake, heart/lung ratio and heart/liver ratio.^{15, 20} In the present study, ^{99m}Tc -15C5-PNP exhibited greater early washout from viable myocardium compared to ^{99m}Tc -sestamibi, although much of the decrease for both agents is attributable to clearance of initial blood-pool activity. Both agents approached a steady state within 10–15 minutes post-injection, with no significant additional washout, and the retained level of ^{99m}Tc -sestamibi was higher throughout. The occurrence of myocardial infarction altered the kinetic profile of both radiotracers in the necrotic myocardium, as expected. Following clearance of blood-pool activity, only a very low level of activity was present within the infarct areas, consistent with scatter from adjacent tissue. Radioactive defects in the hearts with infarcts could be identified by about 15 minutes post-injection with both agents on FastSPECT II imaging.

The activity of ^{99m}Tc -15C5-PNP in the liver declined rapidly, with the result that the entire wall of the left ventricle could be well visualized by 15–20 minutes without interference from the liver. In contrast, ^{99m}Tc -sestamibi liver activity was prominent throughout the 60 minutes after injection. The results of nonlinear liver curve fitting demonstrated that ^{99m}Tc -15C5-PNP was washed out of liver 20 times faster than ^{99m}Tc -sestamibi. The heart/liver ratio of ^{99m}Tc -15C5-PNP determined by biodistribution measurements in this study was about 1.9 times higher than that for ^{99m}Tc -sestamibi at 60 minutes. Thus, the ability of ^{99m}Tc -15C5-PNP to define and quantify activity in the inferior wall early after injection is an advantage over ^{99m}Tc -sestamibi. However, liver washout of ^{99m}Tc -15C5-PNP might not be as rapid as that reported for ^{99m}Tc -N-DBODC5, in which the heart/liver ratio of ^{99m}Tc -N-DBODC5 is about 7 times higher than that of ^{99m}Tc -sestamibi.¹² The liver washout pattern of ^{99m}Tc -15C5-PNP appears similar to that reported for ^{99m}Tc -N-MPO,¹⁸ in which the liver activity of ^{99m}Tc -N-MPO was about 3.5 times lower than that of ^{99m}Tc -15C5-PNP.

The *ex vivo* myocardial activity of ^{99m}Tc -15C5-PNP at 60 minutes post-injection was 1.25 ± 0.09 (%ID/g) in the present study, which is not only lower than results reported for ^{99m}Tc -N-MPO but also inconsistent with biodistribution measurements reported previously.¹⁵ There is some concern that relatively lower myocardial accumulation of ^{99m}Tc -15C5-PNP might bring

down overall image quality. This possible shortcoming might be overcome, if necessary, by moderately lengthening image acquisition time to collect more counts for image reconstruction. In fact, the myocardial images of ^{99m}Tc -15C5-PNP in this study were comparable to those of ^{99m}Tc -sestamibi, using the same acquisition time, and longer imaging times may not be necessary.

Kinetic data showed differences in myocardial retention between ^{99m}Tc -15C5-PNP and ^{99m}Tc -sestamibi, but no significant qualitative or quantitative differences were observed on autoradiograph images. In addition, the ratios of normal to infarcted tissue activity on autoradiograph images were lower than expected from FastSPECT II image data for both tracers. The lack of observed differences may be caused by technical factors. The cardiac tissue slices were stained with TTC prior to autoradiograph imaging. We have noted recently that incubation with TTC buffer can significantly reduce the activity of myocardial perfusion imaging agents in the myocardium. As a result, the differences between radioactive distributions in non-ischemic myocardium and ischemic myocardium were significantly underestimated after TTC staining compared to results before TTC staining.

Conclusion

Good visualization of the normal left ventricular wall and perfusion defects could be achieved 15–20 minutes after intravenous administration of ^{99m}Tc -15C5-PNP in a rat heart model. The quality of myocardial perfusion images with ^{99m}Tc -15C5-PNP was comparable to that with ^{99m}Tc -sestamibi. The perfusion defects in all hearts with infarcts remained well defined for at least 60 minutes post-injection. The liver washout of ^{99m}Tc -15C5-PNP was significantly faster and greater than that of ^{99m}Tc -sestamibi. This improvement of liver kinetics with ^{99m}Tc -15C5-PNP may overcome difficulties in visualizing and quantifying the perfusion of the inferior wall of the left ventricle.

^{99m}Tc -15C5-PNP is a unique $^{99m}\text{Tc}(\text{I})$ -tricarbonyl complex with properties similar to ^{99m}Tc -N-DBODC5 and ^{99m}Tc -N-MPO, and it has potential for rapid myocardial perfusion imaging with low liver uptake. It is worth pursuing preclinical studies with ^{99m}Tc -15C5-PNP using additional animal models.

Acknowledgments

This work was supported by NIH grant P41 EB002035. The authors have indicated they have no financial conflicts of interest.

References

1. Beller GA, Sinusas AJ, Watson DD. Assessment of myocardial perfusion and viability with technetium-99m methoxyisobutyl isonitrile. *Trans Am Clin Climatol Assoc* 1991;102:41–51. [PubMed: 2130569]
2. Glover DK, Ruiz M, Yang JY, Smith WH, Watson DD, Beller GA. Myocardial ^{99m}Tc -tetrofosmin uptake during adenosine-induced vasodilatation with either a critical or mild coronary stenosis: comparison with 201Tl and regional myocardial blood flow. *Circulation* 1997;96(7):2332–8. [PubMed: 9337208]
3. Clark AN, Beller GA. The present role of nuclear cardiology in clinical practice. *Q J Nucl Med Mol Imaging* 2005;49(1):43–58. [PubMed: 15724135]
4. Cherng SC, Chen YH, Lee MS, Yang SP, Huang WS, Cheng CY. Acceleration of hepatobiliary excretion by lemon juice on ^{99m}Tc -tetrofosmin cardiac SPECT. *Nucl Med Commun* 2006;27(11):859–64. [PubMed: 17021425]
5. Garcia EV, Cooke CD, Van Train KF, et al. Technical aspects of myocardial SPECT imaging with technetium-99m sestamibi. *Am J Cardiol* 1990;66(13):23E–31E.

6. van Dongen AJ, van Rijk PP. Minimizing liver, bowel, and gastric activity in myocardial perfusion SPECT. *J Nucl Med* 2000;41(8):1315–7. [PubMed: 10945520]
7. Hurwitz GA, Clark EM, Slomka PJ, Siddiq SK. Investigation of measures to reduce interfering abdominal activity on rest myocardial images with Tc-99m sestamibi. *Clin Nucl Med* 1993;18(9):735–41. [PubMed: 8403714]
8. Iqbal SM, Khalil ME, Lone BA, Gorski R, Blum S, Heller EN. Simple techniques to reduce bowel activity in cardiac SPECT imaging. *Nucl Med Commun* 2004;25(4):355–9. [PubMed: 15097809]
9. Llauro JG. The quest for the perfect myocardial perfusion indicator...still a long way to go. *J Nucl Med* 2001;42(2):282–4. [PubMed: 11216527]
10. Boschi A, Bolzati C, Uccelli L, et al. A class of asymmetrical nitrido ^{99m}Tc heterocomplexes as heart imaging agents with improved biological properties. *Nucl Med Commun* 2002;23(7):689–93. [PubMed: 12089492]
11. Kim YS, He Z, Hsieh WY, Liu S. Impact of bidentate chelators on lipophilicity, stability, and biodistribution characteristics of cationic ^{99m}Tc -nitrido complexes. *Bioconjug Chem* 2007;18(3):929–36. [PubMed: 17352455]
12. Hatada K, Riou LM, Ruiz M, et al. ^{99m}Tc -N-DBODC5, a new myocardial perfusion imaging agent with rapid liver clearance: comparison with ^{99m}Tc -sestamibi and ^{99m}Tc -tetrofosmin in rats. *J Nucl Med* 2004;45(12):2095–101. [PubMed: 15585487]
13. Liu S. Ether and crown ether-containing cationic ^{99m}Tc complexes useful as radiopharmaceuticals for heart imaging. *Dalton Trans* 2007(12):1183–93.
14. Hatada K, Ruiz M, Riou LM, et al. Organ biodistribution and myocardial uptake, washout, and redistribution kinetics of Tc-99m N-DBODC5 when injected during vasodilator stress in canine models of coronary stenoses. *J Nucl Cardiol* 2006;13(6):779–90. [PubMed: 17174809]
15. He Z, Hsieh WY, Kim YS, Liu S. Evaluation of novel cationic ^{99m}Tc (I)-tricarbonyl complexes as potential radiotracers for myocardial perfusion imaging. *Nucl Med Biol* 2006;33(8):1045–53. [PubMed: 17127179]
16. Bolzati C, Cavazza-Ceccato M, Agostini S, Tokunaga S, Casara D, Bandoli G. Subcellular distribution and metabolism studies of the potential myocardial imaging agent [^{99m}Tc (N)(DBODC)(PNP5)]⁺. *J Nucl Med* 2008;49(8):1336–44. [PubMed: 18632814]
17. Takeda T, Wu J, Lwin TT. ^{99m}Tc -N-DBODC5: a novel myocardial perfusion imaging agent for diagnosis of coronary artery disease, a review. *Recent Pat Cardiovasc Drug Discov* 2006;1(2):161–6. [PubMed: 18221083]
18. 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(4):535–46. [PubMed: 18674722]
19. 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(4):571–9. [PubMed: 19288164]
20. Fang W, Liu Y, Zhu L, Kim YS, Liu S, He ZX. Evaluation of ^{99m}Tc -N-15C5 as a new myocardial perfusion imaging agent in normal dogs and canines with coronary stenosis. *Nucl Med Commun* 2008;29(9):775–81. [PubMed: 18677204]
21. Liu Z, Kastis GA, Stevenson GD, et al. Quantitative analysis of acute myocardial infarct in rat hearts with ischemia-reperfusion using a high-resolution stationary SPECT system. *J Nucl Med* 2002;43(7):933–9. [PubMed: 12097466]
22. Furenlid LR, Wilson DW, Chen YC, Kim H, Pietraski PJ, Crawford MJ, Barrett HH. FastSPECT II: a second-generation high-resolution dynamic SPECT imager. *IEEE Trans Nucl Sci* 2004;51:631–5.
23. Liu S, He Z, Hsieh WY, Kim YS. Evaluation of novel cationic (^{99m}Tc)-nitrido complexes as radiopharmaceuticals for heart imaging: improving liver clearance with crown ether groups. *Nucl Med Biol* 2006;33(3):419–32. [PubMed: 16631092]
24. Zhang WC, Fang W, Li B, Wang XB, He ZX. Experimental study of [^{99m}Tc (PNP5)(DBODC)]⁺ as a new myocardial perfusion imaging agent. *Cardiology* 2009;112(2):89–97. [PubMed: 18583906]

Fig 1. Representative FastSPECT II cardiac images (a series of transaxial tomographic slices) of ^{99m}Tc -15C5-PNP (Left panel) and ^{99m}Tc -sestamibi (Right panel) in control rat hearts 60 minutes post-injection with 5-min acquisition. The wall of the left ventricle was fully visible with homogeneous radioactive distribution.

Fig 2. Representative FastSPECT II cardiac images (a series of transaxial tomographic slices) of ^{99m}Tc -15C5-PNP and ^{99m}Tc -sestamibi in rat hearts with myocardial ischemia 60 minutes post-injection with 5-min acquisition. Focal radioactive defects in the lateral and anterior wall of the left ventricle were observed.

Fig 3. Postmortem assays of representative ischemic hearts with ^{99m}Tc -sestamibi and ^{99m}Tc -15C5-PNP. The localization of perfusion defects on autoradiograph images (Top row) were consistent with myocardial ischemic area at risk illustrated by Evans blue (unstained by blue dye) (Middle row) and myocardial infarcts determined by TTC staining (unstained by TTC) (Bottom row).

Fig 4.

Representative dynamic tomographic images of ^{99m}Tc -sestamibi (Left panel) and ^{99m}Tc -15C5-PNP (Right panel) using one selected coronal slice at each time point in healthy control rats. The number in the upper left corner represents the post-injection time. The cardiac blood pool is evident on the 1-minute image. The left ventricular wall becomes increasingly identical from 2 to 60 minutes post-injection of ^{99m}Tc -sestamibi or ^{99m}Tc -15C5-PNP. Relative to quick washout and lower residue of ^{99m}Tc -15C5-PNP in the liver, ^{99m}Tc -sestamibi radioactivity in the liver remained higher level and showed no significant washout within 60 minutes.

Fig 5. ^{99m}Tc -sestamibi (Left panel) and ^{99m}Tc -15C5-PNP (Right panel) myocardial time-activity curves from healthy control rat hearts and ischemic hearts. The curves were corrected by decay, acquisition time. The difference between the radioactivities of ischemic and remote viable zone was significantly different from 5 minutes to 60 minutes in ^{99m}Tc -sestamibi and ^{99m}Tc -15C5-PNP ($P < 0.001$).

Fig 6. Kinetic profiles of ^{99m}Tc -sestamibi and ^{99m}Tc -15C5-PNP in the liver generated by fitting of raw radioactivities with TableCurve 2D. The curve of ^{99m}Tc -sestamibi was plotted using First Order Intermediate Equation with no equilibrium. The curve of ^{99m}Tc -15C5-PNP was generated using were the First Order Intermediate with Equilibrium. Significantly less washout of ^{99m}Tc -sestamibi is observed in the liver than that of ^{99m}Tc -15C5-PNP during the 1-hour acquisition period.

Table 1

Myocardial tracer fractional washout (% of initial peak)

	Ischemia		Control	
	Ischemic Zone	Remote Zone	LCA Zone	RCA Zone
15C5-PNP	95.3±2.0	57.7±5.6*	55.0±2.6*	58.6±7.9*
Sestamibi	96.8±1.6	20.0±4.5	23.8±12.6	18.1±7.8

*
= $P < 0.01$ Compared To Sestamibi

Table 2

Radiopharmaceutical kinetics of the liver

Tracers	T _p (min)	Peak (%)	T _{1/2} (min)	Equilibrium (%)
15C5-PNP	3.7 ± 0.3*	88.1 ± 3.6	6.4 ± 1.1*	14.5 ± 3.6*
Sestamibi	6.5 ± 0.3	93.1 ± 0.9	124.0 ± 9.6	40.6 ± 9.2

T_p = Time to peak activity; T_{1/2} = washout time to 50% of peak activity;

* = $P < 0.05$ compared to sestamibi

Table 3

Data of biodistribution measurements (%ID/gm)

Tissue	Sestamibi		15C5-PNP	
	Control	Ischemia	Control	Ischemia
Blood	0.03±0.02	0.06±0.01	0.03±0.00	0.07±0.01
Heart	1.93±0.16	2.06±0.15	1.25±0.09*	1.08±0.12 [‡]
Lung	0.36±0.10	0.51±0.08	0.35±0.03	0.39±0.05
Muscle	0.08±0.01	0.12±0.01	0.09±0.01	0.10±0.01
Liver	1.14±0.10	1.21±0.04	0.42±0.03*	0.33±0.03 [‡]
Spleen	0.65±0.08	0.98±0.05	0.39±0.09	0.50±0.14 [‡]
Stomach	1.08±0.31	1.26±0.24	0.50±0.15	0.81±0.33
Intestine	1.49±0.12	2.06±0.23	3.33±0.64	3.54±0.90
Kidneys	3.06±0.35	4.62±0.61	2.40±0.30	3.60±0.62
Heart/Liver	1.82±0.35	1.71±0.14	3.11±0.38*	3.31±0.32 [‡]

* = $P < 0.05$ compared to Control group in Sestamibi;[‡] = $P < 0.05$ Compared Ischemia in Sestamibi