Published in final edited form as:

J Labelled Comp Radiopharm. 2014 July ; 57(9): 584–592. doi:10.1002/jlcr.3221.

Development of Kit Formulations for $^{99m}\text{TcN-MPO}$: A Cationic Radiotracer for Myocardial Perfusion Imaging

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

¹School of Health Sciences, Purdue University, Beijing, 100029, China

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

Abstract

The objective of this study was to develop a kit formulation for $^{99m}\text{TcN-MPO}$ to support its clinical evaluations as a SPECT radiotracer. Radiolabeling studies were performed using three different formulations (two-vial formulation and single-vial formulations with/without SnCl_2) to explore the factors influencing radiochemical purity (RCP) of $^{99m}\text{TcN-MPO}$. We found that the most important factor affecting the RCP of $^{99m}\text{TcN-MPO}$ was the purity of PNP5. $^{99m}\text{TcN-MPO}$ was prepared >98% RCP ($n = 20$) using the two-vial formulation. For single-vial formulations with/without SnCl_2 , β -cyclodextrin (β -CD) is particularly useful as a stabilizer for PNP5. The RCP of $^{99m}\text{TcN-MPO}$ was 95 – 98% using β -CD, but its RCP was only 90 – 93% with γ -CD. It seems that PNP5 fits better into the inner cavity of β -CD, which forms more stable inclusion complex than γ -CD in the single-vial formulations. The results from biodistribution and imaging studies in Sprague-Dawley (SD) rats clearly demonstrated biological equivalence of three different formulations. SPECT data suggested that high quality images could be obtained at 0 – 30 min post-injection without significant interference from the liver radioactivity. Considering the ease for ^{99m}Tc -labeling and high RCP of $^{99m}\text{TcN-MPO}$, the non- SnCl_2 single-vial formulation is an attractive choice for future clinical studies.

Keywords

$^{99m}\text{TcN-MPO}$; radiotracers; myocardial perfusion imaging; formulation development

Introduction

More than 70 million Americans live with cardiovascular diseases, which lead to >910,000 deaths each year. Accurate diagnosis is highly desirable so that appropriate therapy can be given before irreversible damage occurs in patients with coronary artery disease (CAD). Myocardial perfusion imaging (MPI) with radiotracers is a well-established noninvasive method of assessing the coronary blood flow. MPI is capable of identifying regional abnormalities in coronary artery and determining their physiological relevance to

*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.

Conflict of Interest

The authors did not report any conflict of interest.

myocardial function and viability.^{1–7} Despite recent development in echocardiography and coronary CT (computed tomography) angiography, MPI with SPECT (single photon-emission computed tomography) radiotracers remains the only reliable imaging modality for assessment of physiological consequence of coronary stenosis or myocardial infarction, and can be combined exercise with pharmacological stress,^{8,9} particularly with the development of the dedicated ultrafast cardiac camera.^{10–14}

^{99m}Tc has a 6.02 h half-life with the single photon energy of 140 keV. The combination of its nuclear properties and diverse coordination chemistry makes ^{99m}Tc the ideal isotope to develop new perfusion imaging agents. Since the 1980s, intensive research efforts have been directed towards using cationic and neutral ^{99m}Tc complexes (Figure 1) as radiotracers for MPI.^{1,15–18} As a result of these extensive efforts, ^{99m}Tc-sestamibi ($[\text{}^{99\text{m}}\text{Tc}(\text{MIBI})_6]^+$; MIBI = 2-methoxy-2-methylpropylisonitrile) and ^{99m}Tc-tetrofosmin (Figure 1: $[\text{}^{99\text{m}}\text{TcO}_2(\text{tetrofosmin})_2]^+$, tetrofosmin = 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane)) have been approved as commercial radiotracers for SPECT MPI. More than 9 million SPECT MPI studies are performed in the United States alone each year. The overwhelming success of SPECT MPI is due to widespread clinical applications of ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin. It is expected that SPECT MPI will continue to play a major role for nuclear cardiology for many years ahead.

Despite their widespread clinical applications, ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin do not meet the requirements of an ideal perfusion agent, at least in part, due to their high liver uptake, which makes it difficult to obtain high quality images at <30 min post-injection (p.i.) and accurately interpret heart radioactivity in the inferior and left ventricular walls.^{8,9,15–18} In order to overcome this shortcoming, ^{99m}TcN-DBODC5 (Figure 1: $[\text{}^{99\text{m}}\text{TcN}(\text{DBODC})(\text{PNP5})]^+$ (PNP5 = N-ethoxyethyl-N,N-bis[2-(bis(3-methoxypropyl)phosphino)ethyl]amine) and DBODC = N,N-bis(ethoxyethyl)dithiocarbamate) was developed as a new SPECT radiotracer because of its fast liver clearance kinetics.^{19–22} However, a kit formulation is needed for its routine preparation in order to achieve wide-spread clinical utility.²⁰ ^{99m}TcN-MPO (Figure 1: $[\text{}^{99\text{m}}\text{TcN}(\text{mpo})(\text{PNP5})]^+$ (mpo = 2-mercaptopyridine oxide)) is another cationic ^{99m}Tc-nitrido complex with very fast liver clearance kinetics.^{23–28} Studies in Sprague-Dawley (SD) rats showed that ^{99m}TcN-MPO has the heart uptake between that of ^{99m}Tc-sestamibi and ^{99m}TcN-DBODC5, and the heart/liver ratio of ^{99m}TcN-MPO was more than twice that of ^{99m}TcN-DBODC5, and ~4 times higher than that of ^{99m}Tc-sestamibi at 30 min p.i.²³ High heart/liver ratios will allow early acquisition of high-quality images, which would be extremely convenient to patients with known or suspected CAD. ^{99m}TcN-MPO is a promising SPECT radiotracer.

Previously, it was shown that ^{99m}TcN-MPO could be prepared with RCP being 90 – 93% using the two-step synthesis and the radio-impurities were mainly caused by oxidation of PNP5 during preparation, storage and ^{99m}Tc-labeling.²³ To support future clinical evaluations of ^{99m}TcN-MPO, this study sought to explore the factors (^{99m}Tc-labeling conditions, stabilizer and the amount of components in each lyophilized vial) influencing its radiochemical purity (RCP). The focus of this study was to stabilize PNP5 with cyclodextrins (CDs). γ -CD has been an excipient and stabilizer for bisphosphines in kit formulations of radiopharmaceuticals,^{19–21,29,30} some of which have been widely used for

MPI studies in humans.²⁹ The β -CD derivatives were found to be useful for stabilization of monosulfonated triphenylphosphine.³¹ The objective of this study was to develop a single-vial kit formulation for the widespread clinical availability of $^{99m}\text{TcN-MPO}$, consistency and reproducibility during its routine radiosynthesis.

Results

Two-Vial Formulation

In the two-vial formulation, SnCl_2 was a reductant for $^{99m}\text{TcO}_4^-$, and SDH was a source of the $[\text{}^{99m}\text{Tc}\equiv\text{N}]^{2+}$ core, which was stabilized by PDTA. PNP5 and MPO were chelators to form $^{99m}\text{TcN-MPO}$.²³ γ -CD was used as an excipient for lyophilization and a stabilizer for PNP5. Sodium phosphate ($\text{Na}_2\text{HPO}_4\cdot\text{H}_2\text{O}$ and NaH_2PO_4) was a buffering agent to control the pH during manufacturing and ^{99m}Tc -labeling. $^{99m}\text{TcN-MPO}$ was prepared in two-steps according to Chart I. First, $^{99m}\text{TcO}_4^-$ was allowed to react with SDH in the presence of excess PDTA and SnCl_2 to afford the ^{99m}Tc -nitrido intermediate, which was allowed to react with a mixture of PNP5 and MPO at 100 °C to afford $^{99m}\text{TcN-MPO}$ in high yield and RCP (>98% and $n = 20$) with minimal amount of $[\text{}^{99m}\text{Tc}]$ colloid (<0.5%). It was found that the chemical purity of PNP5 was critically important in order to achieve high RCP for $^{99m}\text{TcN-MPO}$. Its RCP remained relatively constant as long as PNP5 (1 – 10 mg per vial) was not oxidized during its synthesis, preparation of stock solutions, vial dispensing, lyophilization, storage and ^{99m}Tc -labeling. Oxidization of PNP5 would result in many ^{99m}Tc -containing impurities (Figure 2: small radiometric peaks before and/or after the main peak at ~15 min due to $^{99m}\text{TcN-MPO}$). We also found that β -CD could be used to replace γ -CD without changing the yield and RCP of $^{99m}\text{TcN-MPO}$. Once $^{99m}\text{TcN-MPO}$ was formed, it was able to remain stable in kit matrix for more than 8 h post-labeling at ambient temperature (Figure 2B).

SnCl_2 -Containing Single-Vial Formulation

In this formulation, all components (SnCl_2 , SDH, PDTA, PNP5, MPO, β -CD, $\text{Na}_2\text{HPO}_4\cdot\text{H}_2\text{O}$ and NaH_2PO_4) were lyophilized in a single vial. ^{99m}Tc -labeling was completed in one-step by simply adding $^{99m}\text{TcO}_4^-$ solution and heating the reaction mixture at 100 °C for 10 min (Chart I). The RCP was 95 – 98% ($n = 10$) depending on the total radioactivity in each vial (370 – 1110 MBq). Higher radioactivity levels often resulted in more radio-impurities (2 – 5%), as evidenced by the presence of multiple peaks before and after the main 15-min peak from $^{99m}\text{TcN-MPO}$. The amount of PNP5 and MPO in each lyophilized vial had very little impact on the RCP of $^{99m}\text{TcN-MPO}$ when they were used at the scale of 1 – 10 mg per vial. The presences of air in the reconstituted vials also had little impact on RCP of $^{99m}\text{TcN-MPO}$ as long as β -CD was used as the stabilizer, and PNP5 was not exposed to air for a long time during vial reconstitution and/or ^{99m}Tc -labeling. $^{99m}\text{TcN-MPO}$ from the single-vial formulation (Figure 3A) was chemically equivalent to that from the two-vial formulation.

Non- SnCl_2 Single-Vial Formulation

This formulation is identical to that above except that PNP5 was used as both a reducing agent for $^{99m}\text{TcO}_4^-$ and a co-ligand in $^{99m}\text{TcN-MPO}$. ^{99m}Tc -labeling was completed by

adding 1.0 mL of $^{99m}\text{TcO}_4^-$ solution and heating the reaction mixture at 100 °C for 10 – 15 min (Chart I). The RCP of $^{99m}\text{TcN-MPO}$ was 95 – 98% with no detectable [^{99m}Tc]colloid. It is well-documented that triarylphosphines and trialkylphosphines are excellent reducing agents for $^{99m}\text{TcO}_4^-$.^{33–38} Actually, TPPTS (trisodium triphenylphosphine-3,3',3''-trisulfonate) were used for preparation of $^{99m}\text{TcN-NOET}$ ($^{99m}\text{TcN}(\text{NOET})_2$: NOET = N-ethoxy-N-ethylthiocarbamate) and $^{99m}\text{TcN-DBODC5}$.^{19–21,33–38} $^{99m}\text{TcN-MPO}$ prepared from this formulation had identical HPLC retention time (Figure 3B) to that from the SnCl_2 -containing single-vial formulation.

β -Cyclodextrin versus γ -Cyclodextrin

CDs are cyclic oligosaccharides consist of (α -1,4)-linked α -D-glucopyranose units, and are used as excipients in pharmaceutical formulations.^{39–41} Natural α -, β - and γ -CDs consist of 6, 7, and 8 glucopyranose units, respectively. Due to the chair conformation of glucopyranose units, CDs are shaped like a truncated cone rather than cylinders (Chart II). The central cavity is lined by lipophilic skeletal carbons and ethereal oxygens of glucose residues. CDs can interact with appropriately sized molecules to result in the formation of inclusion complexes (Chart II), which offer significant advantages over the unmanipulated drug substances, including the increased water solubility and solution stability. Since it has better water solubility than β -CD (~20 mg/mL in 0.1 M phosphate buffer), γ -CD (>250 mg/mL in 0.1 M phosphate buffer) is preferred in the pharmaceutical and radiopharmaceutical formulations.^{19–21,29,30} In this study, we found that β -CD is much better than γ -CD for stabilization of PNP5 in single-vial formulations with/without SnCl_2 , as evidenced by lower RCP of $^{99m}\text{TcN-MPO}$ with γ -CD. Using β -CD (~20 mg/vial), the RCP of $^{99m}\text{TcN-MPO}$ was 95 – 98% with/without removal of air (Figure 3). Its RCP was only 90 – 93% using γ -CD (Figure 4). The radioimpurities were mainly from the small radiometric peaks at 11 – 12 min (3 – 8% depending on the total radioactivity level in each reconstituted vial) in its HPLC chromatograms (Figure 4).

Biodistribution Properties

Table 1 lists the selected 30-min biodistribution data of $^{99m}\text{TcN-MPO}$ to compare the biological performance of three formulations. The 30-min biodistribution data were chosen because the radioactivity accumulation in the blood and muscle was low at this time point. Clearly, all three formulations afforded $^{99m}\text{TcN-MPO}$ with very similar uptake values in most organs (Table 1), which strongly suggested that $^{99m}\text{TcN-MPO}$ from three different formulations was biologically equivalent. It is important to note that the biodistribution data and T/B ratios of $^{99m}\text{TcN-MPO}$ described in this study were slightly different from those presented in our previous reports,^{23,24} wherein $^{99m}\text{TcN-MPO}$ was purified before being used for biodistribution studies. Of course, future clinical studies will use $^{99m}\text{TcN-MPO}$ to be prepared from the kit formulation without any chromatographic purification.

Planar Imaging Data

Planar imaging was performed to further compare biological performance of three formulations. Figure 5 shows the whole-body anterior images of SD rats administered with $^{99m}\text{TcN-MPO}$ at 15, 30 and 60 min p.i. Clearly, all the 15-min images showed a relatively low uptake in the liver. By 30 min p.i., the liver radioactivity accumulation was

nearly undetectable while its heart radioactivity remained high. Figure 6 shows the myocardial retention and liver clearance curves of $^{99m}\text{TcN-MPO}$ prepared from three different formulations. A slight difference was seen between the two-vial formulation and the single-vial formulations with/without SnCl_2 , most likely due to their small variations in the RCP of $^{99m}\text{TcN-MPO}$. However, this difference was not significant with experimental errors, as indicated by overlapping of the heart retention and liver clearance curves. The results from image quantification were in good agreement with those reported in our previous study.²³ Both planar imaging and quantification data supported our conclusion that $^{99m}\text{TcN-MPO}$ from three formulations is biologically equivalent.

SPECT Imaging Data

SPECT studies were performed to explore the time window for data acquisition and to further compare biological performance of the two-vial formulation and the non- SnCl_2 single-vial formulation. Figure 6 shows the SPECT images of SD rats administered with $^{99m}\text{TcN-MPO}$ from these two formulations. SPECT images were acquired at 0 – 30 min and 30 – 60 min after administration of $^{99m}\text{TcN-MPO}$. In all cases, the quality of heart images (coronal, sagittal and transaxial) was high with a clear delineation of left and right ventricular walls. The separation between different parts of myocardium was seen due to high resolution of the U-SPECT-II. Because of the fast liver clearance of $^{99m}\text{TcN-MPO}$, there was little significant interference from the liver radioactivity. SPECT data strongly suggested that $^{99m}\text{TcN-MPO}$ from both formulations is biologically equivalent.

DISCUSSION

Discovery of a new ^{99m}Tc radiotracer is just the first step of a long development process. Since ^{99m}Tc is obtained from ^{99}Mo - ^{99m}Tc generators as $\text{Na}^{99m}\text{TcO}_4$ in saline, synthesis of ^{99m}Tc radiotracer must be performed in aqueous solution, and completed within 30 min due to its short half-life (~6 h). Since radiotracers are administered by intravenous injection, radiosynthesis has to be performed under sterile conditions. The RCP of radiotracer must be 95% with high solution stability. Otherwise, post-labeling purification is needed to remove the radioimpurities. Injection of a mixture of ^{99m}Tc species will decrease organ specificity. In this study, we show that $^{99m}\text{TcN-MPO}$ can be prepared from three different formulations. In all cases, the purity of PNP5 was the most important factor influencing the RCP of $^{99m}\text{TcN-MPO}$. The impurities are caused by oxidation of PNP5 during synthesis, storage, vial reconstitution and ^{99m}Tc -labeling. Since PNP5 is sensitive to oxygen, removal of air in reconstituted vials is highly recommended.

A significant advantage of two-vial formulation is the high RCP (>98%) of $^{99m}\text{TcN-MPO}$. However, radiosynthesis with this formulation is more time consuming (>30 min). In contrast, $^{99m}\text{TcN-MPO}$ could be readily prepared in ~10 min with slightly lower RCP (95 – 98%) using single-vial formulations. The advantage of non- SnCl_2 single-vial formulation is the use of PNP5 as a reducing agent. The non- SnCl_2 formulation is preferred in order to minimize ^{99m}Tc colloid formation. The RCP of $^{99m}\text{TcN-MPO}$ from the non- SnCl_2 formulation is comparable to that from the SnCl_2 -containing single-vial formulation (Figure 3). $^{99m}\text{TcN-MPO}$ from three different formulations is chemically identical, as illustrated by

its identical HPLC retention times, and biologically equivalent since $^{99m}\text{TcN-MPO}$ prepared from three different formulations has almost identical biodistribution properties (Table 1) and the same imaging quality (Figure 5).

The results from this study also show that β -CD is a better stabilizer for PNP5 than γ -CD in the single-vial formulations with/without SnCl_2 . Using β -CD, $^{99m}\text{TcN-MPO}$ could be prepared in 95 – 98% RCP while its RCP is only 90 – 93% using γ -CD under the same experimental conditions. The impact of cyclodextrins (β -CD vs. γ -CD) on the RCP of $^{99m}\text{TcN-MPO}$ can be explained in terms of their inner cavity sizes. It seems that PNP5 fits better into the inner lipophilic cavity of β -CD (6.0 – 6.5 Å) as compared to that of γ -CD (7.5 – 8.3 Å),^{40,41} thereby forming the more stable inclusion complex with β -CD (Chart II). As a result, PNP5 is better stabilized by β -CD during preparation of stock solutions, vial dispensing, lyophilization, and ^{99m}Tc -labeling (Figure 4).

The identity of radioimpurities remains unknown. Since the RCP of $^{99m}\text{TcN-MPO}$ from the two-vial formulation is 2 – 3% higher than that from the single-vial formulations with/without SnCl_2 , it is reasonable to believe that these multiple ^{99m}Tc -containing species are originated during reduction of $^{99m}\text{TcO}_4^-$. Oxidation of PNP5 involves extraction of oxygen from $^{99m}\text{TcO}_4^-$. Bonding of the oxidized PNP5 to $^{99m}\text{Tc(V)}$ will result in many ^{99m}Tc -containing species because it might be monodentate with phosphine-P as the donor atom, bidentate with phosphine-P and amine-N atoms or tridentate with phosphine-P, amine-N and phosphine-oxide-O atoms in bonding to $^{99m}\text{Tc(V)}$.

Radiosynthesis of $^{99m}\text{TcN-MPO}$ represents an excellent example of ^{99m}Tc -centered one-pot synthesis from multi-components ($^{99m}\text{TcO}_4^-$, SnCl_2 , SDH, PNP5 and MPO). Unlike traditional organic synthesis, which may involve formation of multiple C-C and C-heteroatom bonds, the one-pot synthesis of $^{99m}\text{TcN-MPO}$ is performed at the tracer level (10^{-7} – 10^{-6} M), and results in formation of multiple coordination bonds between ^{99m}Tc and donor atoms (N, O, S and P) in a single step. ^{99m}Tc -Labeling is accomplished by simply adding $^{99m}\text{TcO}_4^-$ into a kit formulation, and heating the reaction mixture for 10 min. During ^{99m}Tc -labeling, the $^{99m}\text{Tc(VII)}$ in $^{99m}\text{TcO}_4^-$ is reduced to $^{99m}\text{Tc(V)}$ to generate the ^{99m}Tc -nitrido intermediate (Chart I), which undergoes rapid ligand exchange with PNP5 and MPO to afford $^{99m}\text{TcN-MPO}$ in high purity (RCP >95%) without post-labeling chromatographic purification. Since ^{99m}Tc -labeling is performed under sterile conditions, $^{99m}\text{TcN-MPO}$ can be used directly for intravenous injection into patients.

SnCl_2 is a common reducing agent for $^{99m}\text{TcO}_4^-$ in radiopharmaceutical formulations. SDH and PDTA are key components for preparation of $^{99m}\text{TcN-NOET}$ and $^{99m}\text{TcN-DBODC5}$,^{19–21,33–38} both of which have been used in humans. β -CD and γ -CD are recognized as safe pharmaceutical and radiopharmaceutical excipients.^{30,31,39–41} Since PNP5 and MPO are used in such a small amount (1 – 2 mg per vial), it is reasonable to believe that their toxicity will be minimal. This statement is completely consistent with the fact that administration of part of the kit matrix causes no significant adverse effects (vomiting, short breath or death) in animals used for SPECT studies, in which each animal was injected up to 350 – 550 MBq of $^{99m}\text{TcN-MPO}$ (equivalent to one-third or half of the whole kit or more than 120x of the human dose for a 75-kg patient administered with 925

MBq of $^{99m}\text{TcN-MPO}$). Therefore, these three formulations are all amendable for wide clinical applications.

It is interesting to note that the high quality SPECT images could be acquired at 0 – 30 min after administration of $^{99m}\text{TcN-MPO}$ due to its fast liver clearance. This suggests that the same imaging protocol might be used with $^{99m}\text{TcN-MPO}$ in clinical settings. The quality of SPECT images of the rat heart is better than those obtained using micro-PET in the same animal model with $^{18}\text{F-BMS747158-02}$,^{42–44} which is currently under clinical evaluations as a PET radiotracer for MPI.⁴⁵ Despite its low sensitivity (>370 MBq required to obtain high quality gated SPECT images), U-SPECT-II is an excellent imaging platform to evaluate ^{99m}Tc perfusion radiotracers in small animals, such as SD rats.

Conclusions

In conclusion, the major findings of this study are: (1) $^{99m}\text{TcN-MPO}$ could be prepared in high yield and radiochemical purity (RCP >95%) from all three different formulations (two-vial formulation, and single-vial formulations with/without SnCl_2); (2) β -CD is a better stabilizer than γ -CD for PNP5 in the single-vial formulations with/without SnCl_2 ; (3) $^{99m}\text{TcN-MPO}$ from three formulations is chemically identical and biologically equivalent; and (4) high quality SPECT images can be obtained at 0 – 30 min p.i. without significant interference from the liver radioactivity accumulation in SD rats administered with $^{99m}\text{TcN-MPO}$. The combination of easy ^{99m}Tc -labeling with high RCP for $^{99m}\text{TcN-MPO}$ makes the non- SnCl_2 single-vial formulation an attractive choice for future clinical studies.

Experimental

Materials

β -Cyclodextrin (β -CD), γ -cyclodextrin (γ -CD), 1,2-diaminopropane-N,N,N',N'-tetraacetic acid (PDTA), sodium 2-mercaptopyridine N-oxide (MPO), stannous chloride dihydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) and succinic dihydrazide (SDH) were purchased from Sigma/Aldrich (St. Louis, MO). PNP5 (N-ethoxyethyl-N,N-bis[2-(bis(3-methoxypropyl)phosphino)ethyl]amine) was prepared with >98% purity (on the basis of ^{31}P NMR) according to the literature method.²⁸ $\text{Na}^{99m}\text{TcO}_4$ was obtained from Cardinal HealthCare® (Chicago, IL).

Radio-HPLC and ITLC Methods

The HPLC (high performance liquid chromatography) method used an 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×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 NH_4OAc 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 20 min. The RCP for $^{99m}\text{TcN-MPO}$ was calculated as the percentage of peak area over the total area. ITLC (instant thin layer chromatography) used Gelman Sciences silica-gel strips and a 1:1 mixture of acetone/saline (v:v) as eluent. $^{99m}\text{TcN-MPO}$ and $^{99m}\text{TcO}_4^-$ 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.

Preparation of $^{99m}\text{TcN-MPO}$ from Two-Vial Formulation

The two-vial kit formulation is composed of two lyophilized vials (**A** and **B**), where vial **A** contains 5 mg SDH, 5 mg PDTA, 0.1 mg $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, 3.1 mg $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, and 10.9 mg NaH_2PO_4 in freeze-dried form, and vial **B** contains 2 mg PNP5, 2 mg MPO, and 20 mg γ -CD also in freeze-dried form. To vial **A** was added 1.0 mL of $^{99m}\text{TcO}_4^-$ solution (370 – 1850 MBq). The reconstituted vial **A** was kept at room temperature for ~15 min to form the ^{99m}Tc -nitrido intermediate. To vial **B** was added 1.0 mL saline, followed with sonication to make sure that all components were dissolved. The liquid in vial **B** was completely transferred into vial **A**. The resulting mixture was then heated in a boiling water-bath for 10 min. After radiolabeling, vial **A** was allowed to stand at room temperature for 5 min. A sample of the resulting solution was diluted to 185 – 370 Bq/mL with saline, and was then analyzed by radio-HPLC and ITLC.

Preparation of $^{99m}\text{TcN-MPO}$ from SnCl_2 -Containing Single-Vial Formulation

Each lyophilized vial contains 5 mg SDH, 5 mg PDTA, 0.1 mg $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, 2 mg PNP5, 1 mg MPO, 20 mg β -CD, 3.1 mg $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, and 10.9 mg NaH_2PO_4 . To a lyophilized vial was 1.0 mL $^{99m}\text{TcO}_4^-$ solution (370 – 1110 MBq). The reconstituted vial was then heated at 100 °C for 10 – 15 min. After radiolabeling, a sample of the resulting solution was diluted to 185 – 370 Bq/mL with saline, and was then analyzed by radio-HPLC and ITLC.

Preparation of $^{99m}\text{TcN-MPO}$ from Non- SnCl_2 Single-Vial Formulation

Each lyophilized vial contains 5 mg SDH, 5 mg PDTA, 2 mg PNP5, 1 mg MPO, 20 mg β -CD, 3.1 mg $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$ and 10.9 mg NaH_2PO_4 . To each vial was added 1.0 mL $^{99m}\text{TcO}_4^-$ solution (370 – 1110 MBq). After it was evacuated twice with 5 mL syringe to completely remove air, the vial was then heated at 100 °C for 10 – 15 min. After cooling to room temperature, a sample of the resulting solution was diluted to 185 – 370 Bq/mL, and analyzed by radio-HPLC and ITLC.

Doses Preparation

Doses for biodistribution were prepared by dissolving the $^{99m}\text{TcN-MPO}$ kit solution to a concentration of ~1110 kBq/mL with saline containing 15 – 20 % (w/w) propylene glycol. The resulting dose solution was filtered with a 0.20 μm Millex-LG filter unit before being injected into animals. The injection volume was ~0.1 mL for each animal. Doses for planar imaging studies were made by dissolving the $^{99m}\text{TcN-MPO}$ kit solution to ~370 MBq/mL with saline containing 15 – 20 % (w/w) propylene glycol. The injection volume was 0.25 – 0.50 mL for each animal in the imaging studies.

Animal Preparation

All 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 SD rats (200 – 250 g) were purchased from Harlan (Indianapolis, IN), and were

acclimated for >24 h before being used for biodistribution and imaging studies. The protocols were approved by the Purdue University Animal Care and Use Committee (PACUC).

Biodistribution

Animals were anesthetized with intramuscular injection of a mixture of ketamine (80 mg/kg) and xylazine (19 mg/kg) before being used for planar imaging and biodistribution studies. Eight SD rats (4 females and 4 males) were selected. Each animal was administered with 100 – 110 kBq of $^{99m}\text{TcN-MPO}$ via the tail vein. Animals were sacrificed by sodium pentobarbital overdose (100 – 200 mg/kg) at 30 min p.i. Blood was withdrawn from the heart. Organs of interest (heart, brain, lung, liver, spleen, kidneys, muscle and intestine) were excised, rinsed with saline, weighed and counted on a Perkin Elmer Wizard – 1480 γ -counter (Shelton, CT). Organ uptake was calculated as the percentage of injected dose per gram (%ID/g). Biodistribution data and T/B ratios were reported as an average $\pm$ standard deviation on the basis of results from 8 animals in each group unless specified. Comparison between two formulations was made using a one-way ANOVA test. The level of significance was set at $p < 0.05$.

Planar Imaging

Five female SD rats (200 – 250 g) were used for planar imaging studies. Animals were anesthetized with intramuscular injection of a mixture of ketamine (80 mg/kg) and xylazine (19 mg/kg) before being used for planar imaging and biodistribution studies. Each animal was administered with $^{99m}\text{TcN-MPO}$ (25 – 50 MBq) via the tail vein injection. The animal was then 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 $\times$ 128 matrix. The count limits were set at 300 K. For dynamic imaging, the 2-min static images were acquired at 0 – 30 min p.i., followed with whole body static images at 40, 50 and 60 min p.i. After imaging, animals were returned to the lead-shielded cage for recovery. Planar images were analyzed by drawing regions of the heart, liver and standard radiation source. The results were expressed as a percentage of the initial radioactivity in that organ. The exponential fit of the heart retention and liver clearance were determined using GraphPad Prim 5.0 (GraphPad Software, Inc., San Diego, CA). The image quantification data were reported as an average plus/minus standard deviation on the basis of results from 5 animals in each group. Comparison between two formulations was made using a one-way ANOVA test. The level of significance was set at $p < 0.05$.

Protocol for EKG-Gated SPECT

Two SD rats were used for electrocardiography (EKG)-gated SPECT studies. One was injected with 350 MBq of $^{99m}\text{TcN-MPO}$ while the other was administered with 550 MBq of $^{99m}\text{TcN-MPO}$ via the tail-vein injection. The EKG-gated SPECT images were obtained using a u-SPECT-II/CT scanner (Milabs, Utrecht, The Netherlands) equipped with a 1.0 mm multi-pinhole collimator. The SD rat was anesthetized by connected to an isoflurane

anesthesia unit (Univentor, Zejtun, Malta) using an air flow rate of 350 mL/min with ~3.0% isoflurane, and was maintained using the air flow rate of ~250 mL/min with ~2.0% isoflurane during the whole time of preparation and data acquisition (2 frames: 75 projections over 30 min per frame). The hair of the entire chest and the inner side of the right hind limb was shaved. A 24G (0.67×19 mm) catheter (Somerset, NJ, USA) was inserted to the tail vein and connected with a 1.0 ml syringe filled with sterilized saline. After the animal was placed supine on the scanning bed, EKG leads were attached to a piece of tape. After the EKG gel was added on each lead, the tape was attached tightly onto the chest or right hind limb to achieve a good electric connection. The gain and threshold parameters were adjusted to make sure that the waveform (not the noise) broke the threshold consistently. Rectangular scan area limited to the region of heart was selected on the basis of orthogonal X-ray images provided by the integrated CT. The SD rat was administered with $^{99m}\text{TcN-MPO}$ dissolved in 0.5 mL saline containing ~20% propylene glycol through the catheter above, followed with 0.5 mL saline solution flush. After SPECT data acquisition, the animal was allowed to recover in a lead-shielded cage.

Image Reconstruction and Data Processing

SPECT reconstruction was performed using a POSEM (pixelated ordered subsets by expectation maximization) on a 0.375 mm isotropic voxel grid with 8 iterations and 12 subsets. The total number of detected photons was determined for each scan in a 20% energy window around 140 keV. For image reconstruction, four frames were used per cardiac cycle. The beat acceptance window was set at the center of the average R-R interval calculated just before starting image acquisition. The width of the heart beat acceptance window was set at 50% of this average R-R interval.³² A 3D-Gaussian filter (1.0 mm FWHM) was applied to smooth noise. The LUTs (look up tables) were adjusted for good visual contrast, the rotate tool in the PMOD software's view tool was used to get the coronal, transaxial and sagittal images.

Acknowledgement

Authors would thank Dr. Aaron B. Taylor for his technical assistance in SPECT/CT studies. 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

CAD	coronary artery disease
β-CD	β-cyclodextrin
γ-CD	γ-cyclodextrin
EDTA	ethylenediamine-N,N,N',N'-tetraacetic acid
MPI	myocardial perfusion imaging
PDTA	1,2-diaminopropane-N,N,N',N'-tetraacetic acid

RCP	radiochemical purity
SDH	succinic dihydrazide
SPECT	single photon-emission computed tomography
^{99m}TcN-DBODC5	[^{99m} TcN(DBODC)(PNP5)] ⁺ (PNP5 = N-ethoxyethyl-N,N-bis[2-(bis(3-methoxypropyl)phosphino)ethyl]amine, and DBODC = N,N-bis(ethoxyethyl)dithiocarbamate)
^{99m}TcN-NOET	^{99m} TcN(NOET) ₂ (NOET = N-ethoxy-N-ethyldithiocarbamate)
^{99m}TcN-MPO	[^{99m} TcN(mpo)(PNP5)] ⁺ (mpo = 2-mercaptopyridine oxide)
^{99m}Tc-sestamibi	[^{99m} Tc(MIBI) ₆] ⁺ (MIBI = 2-methoxy-2-methylpropylisocyanide); and
^{99m}Tc-tetrofosmin	[^{99m} TcO ₂ (tetrofosmin) ₂] ⁺ (tetrofosmin = 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane).

References

- Acampa W, Di Benedetto C, Cuocolo A. J. Nucl. Cardiol. 2000; 7:701–707. [PubMed: 11144485]
- Dilsizian V. J. Nucl. Cardiol. 2000; 7:180–184. [PubMed: 10796009]
- Beller GA, Zaret BL. Circulation. 2000; 101:1465–1478. [PubMed: 10736294]
- Parker JA. Semin. Nucl. Med. 2001; 31:223–237. [PubMed: 11430529]
- Kapur A, Latus KA, Davies G, Dhawan RT, Eastick S, Jarritt PH, Roussakis G, Young MC, Anagnostopoulos C, Bomanji J, Costa DC, Pennell DJ, Prvulovich EM, Ell PJ, Underwood SR. Eur. J. Nucl. Med. Mol. Imaging. 2002; 29:1608–1616. [PubMed: 12458395]
- Henneman MM, Schuijf JD, van der Wall EE, Bax JJ. Br. Med. Bull. 2006; 79–80:187–202.
- Stirrup J, Wechalekar K, Maenhout A, Anagnostopoulos C. Br. Med. Bull. 2009; 89:63–78. [PubMed: 19179343]
- Kailasnath P, Sinusas AJ. J. Nucl. Cardiol. 2001; 8:482–498. [PubMed: 11481571]
- Llaurado JG. J. Nucl. Med. 2001; 42:282–284. [PubMed: 11216527]
- Gaemperli O, Kaufmann PA. J. Nucl. Cardiol. 2011; 18:830–832. [PubMed: 21681614]
- Perrone-Filardi P, Costanzo P, Dellegrottaglie S, Gargiulo P, Ruggiero D, Savarese G, Parente A, D'Amore C, Cuocolo A, Chiariello M. J. Nucl. Cardiol. 2010; 17:310–315. [PubMed: 20033857]
- Esteves FP, Raggi P, Folks RD, Keidar Z, Askew JW, Rispler S, O'Connor MK, Verdes L, Garcia EV. J. Nucl. Cardiol. 2009; 16:927–934. [PubMed: 19688410]
- Schillaci O, Danieli R. Eur. J. Nucl. Med. Mol. Imaging. 2010; 37:1706–1709. [PubMed: 20593283]
- Fiechter M, Ghadri JR, Kuest SM, Pazhenkottil AP, Wolfrum M, Nkoulou RN, Goetti R, Gaemperli O, Kaufmann PA. Eur. J. Nucl. Med. Mol. Imaging. 2011; 38:2025–2030. [PubMed: 21761267]
- Nunn AD. Semin. Nucl. Med. 1990; 20:111–118. [PubMed: 2184519]
- Saha GB, Go RT, MacIntyre WJ. Int. J. Rad. Appl. Instrum. B. 1992; 19:1–20. [PubMed: 1577608]
- Jain D. Semin. Nucl. Med. 1999; 29:221–236. [PubMed: 10433338]
- Banerjee S, Pillai MR, Ramamoorthy N. Semin. Nucl. Med. 2001; 31:260–277. [PubMed: 11710769]
- Boschi A, Uccelli L, Bolzati C, Duatti A, Sabba N, Moretti E, Di Domenico G, Zavattini G, Refosco F, Giganti M. J. Nucl. Med. 2003; 44:806–814. [PubMed: 12732683]

20. Hatada K, Riou LM, Ruiz M, Yamamichi Y, Duatti D, Lima RL, Goode AR, Watson DD, Beller GA, Glover DK. *J. Nucl. Med.* 2004; 45:2095–2101. [PubMed: 15585487]
21. Hatada K, Ruiz M, Riou LM, Lima RL, Goode AR, Watson DD, Beller GA, Glover DK. *J. Nucl. Cardiol.* 2006; 13:779–790. [PubMed: 17174809]
22. Duatti A, Liu S, Pasqualini R. *Curr. Radiopharm.* 2009; 2:268–276.
23. Kim YS, Wang J, Broisat A, Glover DK, Liu S. *J. Nucl. Cardiol.* 2008; 15:535–546. [PubMed: 18674722]
24. Kim YS, Shi J, Zhai S, Hou G, Liu S. *J. Nucl. Cardiol.* 2009; 16:571–579. [PubMed: 19288164]
25. Bu L, Li R, Jin Z, Wen X, Liu S, Yang B, Shen B, Chen X. *Mol. Imaging Biol.* 2011; 13:121–127. [PubMed: 20458635]
26. Liu S. *Dalton Trans.* 2007; 28:1183–1193. [PubMed: 17353949]
27. Kim YS, Wang F, Liu S. *World J. Hepatol.* 2010; 2:21–31. [PubMed: 21160953]
28. Liu S, He Z, Hsieh WY, Kim YS. *Nucl. Med. Biol.* 2006; 33:419–432. [PubMed: 16631092]
29. Hendel RC, Verani MS, Miller DD, Wackers FJ, McMahon M, Cerqueira MD, Botvinick EH, Kvols L, Gerson MC. *J. Nucl. Cardiol.* 1996; 3:291–300. [PubMed: 8799247]
30. Marmion ME, Woulfe SR, Neumann WL, Nosco DL, Deutsch E. *Nucl. Med. Biol.* 1999; 26:755–770. [PubMed: 10628555]
31. Canipelle M, Tilloy S, Ponchel A, Bricout H, Monflier E. *J. Incl. Phenom Macrocycl. Chem.* 2005; 51:79–85.
32. Vanhove C, Defrise M, Franken PR, Everaert H, Deconinck F, Bossuyt A. *Eur. J. Nucl. Med.* 2000; 27:140–146. [PubMed: 10755718]
33. Mallia MB, Mathur A, Subramanian S, Banerjee S, Kothari K, Koiry SP, Sarma HD, Venkatesh M. *Appl. Radiat. Isot.* 2006; 64:361–367. [PubMed: 16293419]
34. Pasqualini R, Duatti A. *J. Chem. Soc. Chem. Commun.* 1992; 18:1354–1355.
35. Duatti A, Pasqualini R. *J. Chem. Soc. Dalton Trans.* 1990; 2:3729–3733.
36. Pasqualini A, Duatti A, Bellande E, Comazzi V, Brucato V, Hoffschir D, Fagret D, Comet M. *J. Nucl. Med.* 1994; 35:334–341. [PubMed: 8295007]
37. Bolzati C, Boschi A, Uccelli L, Tisato F, Refosco F, Cagnolini A, Duatti A, Prakash S, Bandoli G, Vittadini A. *J. Am. Chem. Soc.* 2002; 124:11468–11479. [PubMed: 12236761]
38. Boschi A, Duatti A, Uccelli L. *Top. Curr. Chem.* 2005; 252:85–115.
39. Loftsson T, Brewster ME. *J. Pharm. Sci.* 1996; 85:1017–1025. [PubMed: 8897265]
40. Magnúsdóttir A, Másson M, Loftsson T. *J. Incl. Phenom Macroc. Chem.* 2002; 44:213–218.
41. Del Valle EMM. *Process Biochem.* 2004; 39:1033–1046.
42. Sherif HM, Saraste A, Weidl E, Weber AW, Higuchi T, Reder S, Poethko T, Henriksen G, Casebier D, Robinson SP, Wester HJ, Nekolla SJ, Schwaiger M. *Circ. Cardiovasc. Imaging.* 2009; 2:77–84. [PubMed: 19808572]
43. Yu M, Guaraldi MT, Mistry M, Kagan M, McDonald JL, Drew K, Radeke H, Azure M, Purohit A, Casebier D, Robinson SP. *J. Nucl. Cardiol.* 2007; 14:789–798. [PubMed: 18022105]
44. Yu M, Guaraldi MT, Bozek J, Kagan M, Azure M, Radeke H, Cdebaca M, Robinson SP. *J Nucl Cardiol.* 2009; 16:763–768. [PubMed: 19548049]
45. Maddahi J, Czernin J, Lazewatsky J, Huang SC, Dahlbom M, Schelbert H, Sparks R, Ehlgen A, Crane P, Zhu Q, Devine M, Phelps M. *J. Nucl. Med.* 2011; 52:1490–1498. [PubMed: 21849402]

Figure 1.

Structures of cationic ^{99m}Tc radiotracers: ^{99m}Tc -Sestamibi, ^{99m}TcN -DBODC5 and ^{99m}TcN -MPO. ^{99m}Tc -Sestamibi is the most widely used radiotracer for SPECT MPI. ^{99m}TcN -DBODC5 and ^{99m}TcN -MPO have fast liver clearance kinetics, and both are currently under clinical evaluations as new radiotracers for SPECT MPI.

Figure 2. Solution stability data of $^{99\text{m}}\text{TcN-MPO}$ prepared from the two-vial formulation. The RCP was >98% (n = 20).

Figure 3.

Radio-HPLC chromatograms of ^{99m}TcN-MPO prepared with the SnCl₂-containing single-vial (top: n = 10) and non-SnCl₂ single-vial (bottom: n = 10) formulations. The RCP was 95 – 98%. Many ^{99m}Tc-containing species (2 – 5% radioimpurity depending on the radioactivity level in each reconstituted vial) were formed before and after the peak at 15.5 min from ^{99m}TcN-MPO.

Figure 4.

Typical radio-HPLC chromatograms of $^{99\text{m}}\text{TcN-MPO}$ prepared using the SnCl_2 -containing single-vial (top: $n = 5$) and non- SnCl_2 single-vial (bottom: $n = 5$) formulations containing γ -CD. The RCP was only 90 – 93%. The radioimpurities are radiometric peaks at 11 – 12 min (3 – 8% the total radioactivity).

Figure 5.

Comparison of whole-body planar images of SD rats ($n = 5$) administered with ^{99m}TcN -MPO prepared by three different formulations to show their biological equivalence. Each rat was injected with 0.5 – 2.0 mCi of ^{99m}TcN -MPO. Images were obtained at 15, 30 and 60 min p.i.

Figure 6.

Image quantification data to compare heart retention and liver excretion kinetics of $^{99\text{m}}\text{TcN-MPO}$ prepared from three different formulations. The experimental data were expressed as a percentage of the initial radioactivity at 0 – 2 min p.i.

Figure 7.

Selected EKG-gated SPECT (coronal, transaxial and sagittal) images of the SD rats administered with $^{99m}\text{TcN-MPO}$ prepared from the two-vial (~ 350 MBq) and non- SnCl_2 single-vial (~ 550 MBq) formulations. These images were acquired at 0 – 30 and 30 – 60 min p.i. There was no significant interference from the liver radioactivity due to its fast liver clearance.

Two-Vial Formulation

Single-Vial Formulation With SnCl_2

Single-Vial Formulation Without SnCl_2

Chart I.

Radiosynthesis of $^{99m}\text{TcN-MPO}$ with Two-Vial and Single-Vial Formulations

Chart II.
Schematic Illustration of PNP5 Stabilization with β -CD and γ -CD.

Table 1

Selected 30-min biodistribution data (%ID/g) in SD rats for $^{99m}\text{TcN-MPO}$ prepared from three different formulations.

Organ	Two-Vial Formulation (n = 4)	Single-Vial (SnCl_2) Formulation (n = 8)	Single-Vial (Non- SnCl_2) Formulation (n = 8)
Blood	0.03 ± 0.01	0.09 ± 0.03	0.08 ± 0.02
Brain	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
Heart	2.29 ± 0.07	2.17 ± 0.19	2.23 ± 0.13
Intestines	1.62 ± 0.20	1.48 ± 0.42	2.05 ± 0.55
Kidneys	4.33 ± 1.61	2.58 ± 0.52	3.54 ± 0.60
Liver	0.33 ± 0.05	0.40 ± 0.14	0.46 ± 0.16
Lungs	0.49 ± 0.14	0.53 ± 0.08	0.61 ± 0.15
Muscle	0.24 ± 0.10	0.21 ± 0.09	0.21 ± 0.04
Spleen	0.35 ± 0.03	0.34 ± 0.11	0.37 ± 0.10
Heart/Blood	83.99 ± 13.86	24.89 ± 7.25	29.37 ± 7.26
Heart/Lungs	4.67 ± 0.53	4.32 ± 1.06	4.06 ± 0.39
Heart/Liver	6.86 ± 1.50	6.22 ± 1.52	5.87 ± 0.77
Heart/Muscle	9.36 ± 0.72	11.20 ± 5.04	11.01 ± 2.52