World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.21

World J Hepatol 2010 January 27; 2(1): 21-31
ISSN 1948-5182 (online)

REVIEW

Minimizing liver uptake of cationic ^{99m}Tc radiotracers with ether and crown ether functional groups

Young-Seung Kim, Fan Wang, Shuang Liu

Young-Seung Kim, Shuang Liu, School of Health Sciences, Purdue University, 550 Stadium Mall Drive, West Lafayette, IN 47907, United States
Fan Wang, Medical Isotopes Research Center, Peking University, Beijing 100083, China
Author contritions: Kim YS, Liu S and Wang F contributed equally to this work. All of them were responsible for manuscript writing and revising.
Supported in part by Purdue University and research grants: R01 CA115883 A2 (S.L.) from National Cancer Institute, R21 EB003419-02 (S.L.) from National Institute of Biomedical Imaging and Bioengineering and R21 HL083961-01 from National Heart, Lung, and Blood Institute
Correspondence to: Shuang Liu, PhD, School of Health Sciences, Purdue University, 550 Stadium Mall Drive, West Lafayette, IN 47907, United States. lius@pharmacy.purdue.edu
Telephone: +1-765-4940236 Fax: +1-765-4961377
Received: September 1, 2009 Revised: January 11, 2010
Accepted: January 18, 2010
Published online: January 27, 2010

Abstract

Ischemia-related diseases, particularly coronary artery disease (CAD), account for the majority of deaths worldwide. Myocardial ischemia is a serious condition and the delay in reperfusion of ischemic tissues can be life-threatening. This is particular true in the aged population. Rapid and accurate early detection of myocardial ischemia is highly desirable so that various therapeutic regiments can be given before irreversible myocardial damage occurs. Myocardial perfusion imaging with radiotracers is an integral component in evaluations of patients with known or suspected CAD. ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin are commercial radiopharmaceuticals currently available for myocardial perfusion imaging. Despite their widespread clinical applications, both ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin do not meet the requirements of an ideal perfusion imaging agent, largely due to their high liver uptake. The intense liver uptake makes it difficult to interpret the heart activity in the inferior and left ventricular wall. Photon scattering from the high liver radioactivity accumulation remains a significant challenge for diagnosis of heart diseases. This review will summarize the most recent research efforts to minimize the liver uptake of cationic ^{99m}Tc radiotracers by using ether and crown ether-containing chelators. Fast liver clearance will shorten the duration of imaging protocols (< 30 min post-injection), and allow for early acquisition of heart images with high quality. Improvement of heart/liver ratio may permit better detection of the presence and extent of coronary artery disease. Identification of such a new radiotracer that allows for the improved noninvasive assessment of myocardial perfusion would be of considerable benefit in treatment of patients with suspected CAD.

Key words: Myocardial perfusion imaging; Cationic ^{99m}Tc radiotracers; Single photon emission computed tomography

Peer reviewer: Fei Chen, PhD, Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, United States

Kim YS, Wang F, Liu S. Minimizing liver uptake of cationic ^{99m}Tc radiotracers with ether and crown ether functional groups. *World J Hepatol* 2010; 2(1): 21-31 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/21.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i1.21

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of premature and permanent disability. CAD arises from gradual narrowing of coronary artery due to atherosclerotic deposits. The progressive narrowing of coronary artery

eventually predisposes the patient to myocardial ischemia, a condition in which coronary blood flow decreases to a level below what is needed to meet the demand for oxygen and nutrients. When coronary arterial lumen diameter is reduced by 50%, perfusion abnormalities can be detected but patients are usually asymptomatic. When the diameter is reduced by 70%, clinical symptoms occur during myocardial stress because tissue oxygenation is temporarily below what is needed for adequate heart function. In the advanced ischemic CAD, blood flow and tissue oxygenation are too low to sustain cardiac function at rest. As a result, myocardial infarction occurs. Therefore, rapid and accurate early detection of myocardial ischemia and infarction is highly desirable so that appropriate therapeutic regimens can be given before irreversible myocardial damage occurs.

Diagnostic radiotracers are small molecules labeled with a γ-emitter for single photon emission computed tomography (SPECT) or positron-emitter for positron emission tomography (PET). Nuclear cardiology plays a key role in CAD patient management[1-12]. Precise measurement of regional blood flow has significant clinical importance in identifying myocardial ischemia and infarction, defining the extent and severity of disease, assessing myocardial viability, establishing the need for medical and surgical intervention, and monitoring the effects of treatment. Thus, the radiotracer must be taken up by the myocardium proportionally to the blood flow in order to evaluate areas with reduced blood flow. If the patient has CAD, there will be an area of reduced radiotracer uptake in the myocardium, corresponding to the area of reduced blood flow. If the reduced uptake is worse under stress conditions than that at rest, the perfusion defect is most likely due to ischemia. Information gained during perfusion imaging studies can be used not only to identify CAD but also to give insight into the patient's prognosis, such as the probability of a hard cardiac event (myocardial infarction or cardiac-related death).

Despite recent development of new non-invasive imaging technologies, such as stress echocardiography and coronary CT (computed tomography) angiography, and wider availability of PET, myocardial perfusion imaging with SPECT radiotracers remains the mainstay of non-invasive evaluations in patients with known or suspected coronary artery disease (CAD)[1-9]. It is the only available imaging technology that assesses the physiological consequence of coronary stenosis and can be combined with exercise and pharmacological stress[10].

The introduction of ^{201}Tl in 1970's was the turning point of widespread clinical use of myocardial perfusion imaging, and had a profound impact on therapeutic decision-making in patients with CAD over the last three decades. However, the combination of long half-life ($t_{1/2}$ = 73 h), attenuation artifacts due to low abundance of γ-photons and the low count rate from dose constraints may result in suboptimal images in a significant proportion of perfusion imaging studies using ^{201}Tl. In addition, ^{201}Tl images should be taken as soon as it is injected into the patient due to its distribution and redistribution dynamics, which may not be suitable for situations where immediate imaging is not possible (for example, patients with acute myocardial infarction).

Compared to ^{201}Tl, ^{99m}Tc yields relatively high-energy photons (~140 keV) and can be used at high doses due to its short-half life ($t_{1/2}$ = 6.01 h). The use of ^{99m}Tc allows simultaneous assessment of myocardial perfusion and cardiac function in a single study[11]. The combination of half-life, optimal γ-energy and diverse coordination chemistry makes ^{99m}Tc the isotope of choice for development of myocardial perfusion radiotracers. In early 1980s, intensive efforts were focused on the development of ^{99m}Tc complex radioph armaceuticals[1,2,5,13]. As a result, ^{99m}Tc-Sestamibi, ^{99m}Tc-Tetrofosmin, and ^{99m}Tc-Teboroxime (Figure 1) have been approved as commercial radiopharmaceuticals for myocardial perfusion imaging in nuclear cardiology. These cationic ^{99m}Tc radiotracers are highly lipophilic with cationic or neutral charge, contain at least two ether-like linkages (N-O-R or C-O-R), and are excreted though the hepatobiliary system due to their high lipophilicity.

An ideal perfusion radiotracer should have a high heart uptake with stable myocardial retention, which linearly tracks myocardial blood flow over a wide range. The uptake in the liver and lungs should be minimal so that diagnostically useful images can be obtained within 30 min post-injection. Despite their widespread applications, both ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin do not meet the requirements of an ideal perfusion imaging agent due to their high liver uptake and inability to track the increase in the myocardial blood flow well with roll-off at higher blood flow levels[14]. The intense liver uptake makes it difficult to interpret the heart activity in the inferior and left ventricular wall[1,2,5,12,13]. Because of their enterohepatic clearance, the gut uptake is often aggravated by pharmacological stress[14]. Despite intensive efforts to reduce this interference, photon scattering from the liver activity remains a significant challenge for diagnosis of heart diseases. Thus, it would be of great benefit to develop a new ^{99m}Tc perfusion radiotracer that has high heart uptake with the heart/liver ratio substantially better than that of ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin. This review article will summarize recent research efforts to minimize the liver uptake of cationic ^{99m}Tc radiotracers, and will focus on the use of ether and crown ether groups to improve their liver clearance kinetics. The main objective is to illustrate that minimizing liver radioactivity accumulation is critically important for new ^{99m}Tc radiotracers. The ultimate goal is to develop a new ^{99m}Tc perfusion radiotracer that will satisfy the unmet medical need and serve a large population of patients with known or suspected CAD.

IMPROVING LIVER CLEARANCE BY ETHER GROUPS

The usefulness of ether groups to reduce radiotracer liver uptake was first observed in development of ^{99m}Tc-

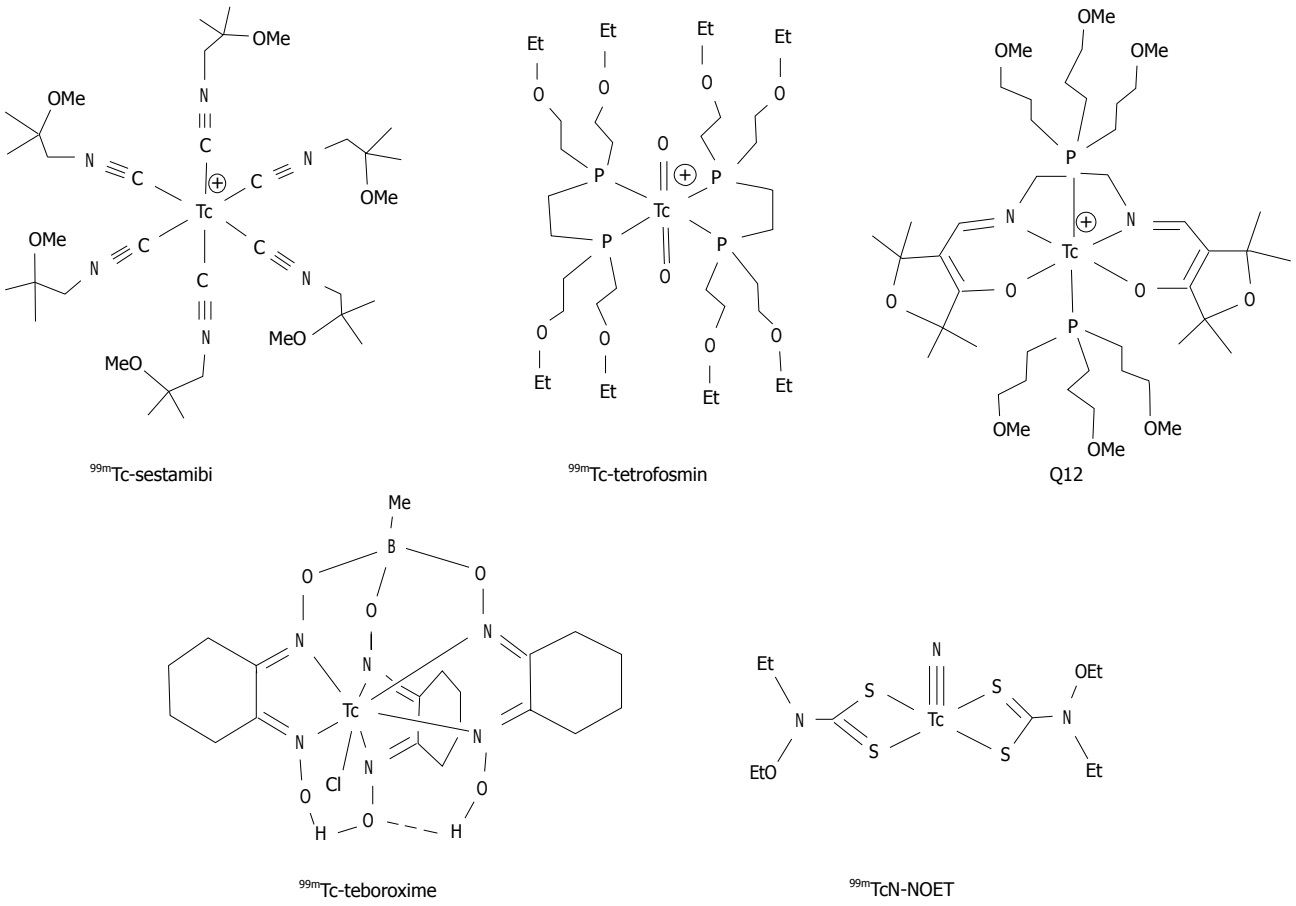

Figure 1 ^{99m}Tc radiotracers useful for heart imaging. ^{99m}Tc-Sestamibi, ^{99m}Tc-Tetrofosmin and ^{99m}Tc-Teboroxime have been approved as commercial radiopharmaceuticals for myocardial perfusion imaging.

Sestamibi[15-18]. Since then, many ether-containing ligands or chelators have been used to improve T/B ratios of cationic ^{99m}Tc complexes[19-22]. For example, studies on Q-series of ^{99m}Tc complexes showed that ethers on phosphine ligands could improve the heart uptake and imaging properties[19]. ^{99m}Tc-Sestamibi, ^{99m}Tc-Tetrofosmin and Q12 all contain six or more ether groups and show much less liver uptake as compared to ^{99m}TcN-Noet and ^{99m}Tc-Teboroxime (Figure 1).

Ether-containing cationic ^{99m}Tc-nitrido complexes.

Duatti's group reported a series of cationic ^{99m}Tc-nitrido complexes (Figure 2), which contain a $[^{99m}Tc\equiv N]^{2+}$ core, a bidentate dithiocarbamate (DTC), a tridentate PNP-type bisphosphine[24,25]. It was found that the amine-N donor atom in the PNP bisphosphine chelator invariably is trans to the Tc≡N triple bond[24,25]. The nitrogen heteroatom is important to provide stabilization for the $[^{99m}Tc\equiv N]^{2+}$ core. It is remarkable that the combination of DTCs and bisphosphines results in formation of cationic ^{99m}Tc-nitrido complexes of high yield and high radiochemical purity[24,25]. Results from animal studies show that DTCs and bisphosphines have significant impact on biodistribution properties of cationic ^{99m}Tc-nitrido complexes[26-29]. Among the cationic ^{99m}Tc-nitrido complexes evaluated in Sprague-Dawley (SD) rats, ^{99m}TcN-DBODC5 (Figure 2) had a high heart uptake and was able to retain in the rat myocardium for more than 2 h[26,27]. The liver radioactivity accumulation was almost completely eliminated at 2 h p.i. with the heart/liver ratios being 10 × better than that of ^{99m}Tc-Sestamibi[26,27]. Imaging studies also showed that ^{99m}TcN-DBODC5 had a fast liver clearance, and was able to give clear images of the heart as early as 15 min post-injection in SD rats[28]. The first-pass extraction fraction of ^{99m}TcN-DBODC5 was between that of ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin[29]. ^{99m}TcN-DBODC5 is currently under clinical investigation as a new myocardial perfusion radiotracer.

Other bidentate chelators have been used to replace DTC ligands in ^{99m}TcN-DBODC[30-33]. The use of these bidentate chelators offers a great structural diversity, and allows easy modification of the lipophilicity and biological properties of cationic ^{99m}Tc radiotracers. For example, 2-mercaptopyridine oxide was used to prepare ^{99m}TcN-MPO (Figure 2). ^{99m}TcN-MPO and ^{99m}TcN-DBODC5 shared the same basic structure, but differ in the bidentate π-donor chelating ligand. They also have very similar in vivo stabilities. Biodistribution studies in SD rats showed that ^{99m}TcN-MPO had a high initial heart uptake (2.45 ± 0.58 %ID/g at 5 min post-injection), with a long myocardial retention (2.44 ± 0.46 %ID/g at

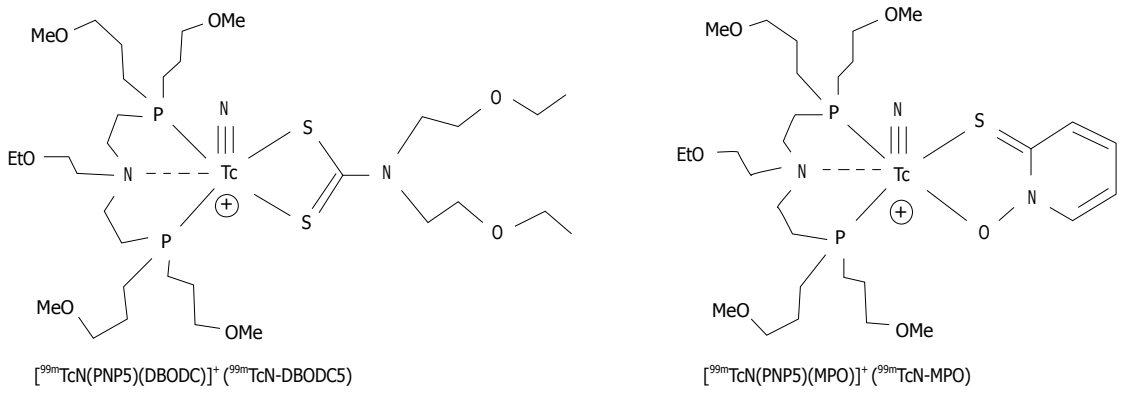

Figure 2 Cationic ^{99m}Tc-nitrido complexes with fast liver clearance and excellent heart/liver ratios. Both ^{99m}TcN-DBODC5 and ^{99m}TcN-MPO are under clinical investigation as new myocardial perfusion imaging agents.

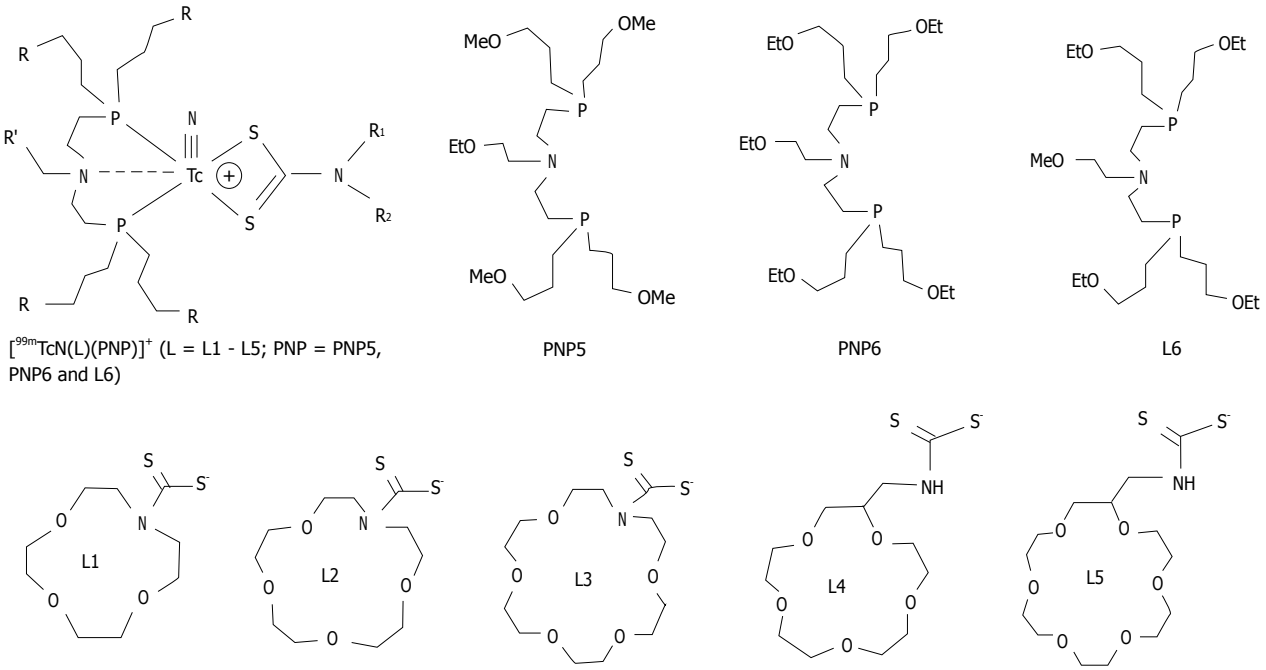

Figure 3 Crown ether-containing DTCs, bisphosphines and their cationic ^{99m}Tc-nitrido complexes.

120 min post-injection)[46]. The heart uptake of ^{99m}TcN-MPO was between that of ^{99m}Tc-sestamibi and that of ^{99m}TcN-DBODC. The liver clearance was fast, resulting in excellent heart/liver ratios. The heart/liver ratio of ^{99m}TcN-MPO at 30 min post-injection was 12.75 ± 3.34, which is ~4 × higher than that of ^{99m}Tc-sestamibi (2.90 ± 0.62) and 2 × higher than that of ^{99m}TcN-DBODC (6.01 ± 1.45). By 120 min post-injection, the heart/liver ratio of ^{99m}TcN-MPO increased to 27.60 ± 8.44, which is 8 × that of ^{99m}Tc-sestamibi (3.52 ± 0.34) and is slightly better than that of ^{99m}TcN-DBODC (21.20 ± 3.39) at the same time point, although this difference is not significant within the experimental error.

Crown ether-containing cationic ^{99m}Tc-nitrido complexes

The crown ether-containing DTCs (Figure 3) are of particular interest because they are able to form stable cationic ^{99m}Tc-nitrido complexes in combination with bisphosphines, and the crown ether groups are able to balance the lipophilicity of cationic ^{99m}Tc-nitrido complexes without changing their overall molecular charge[31,32]. Both crown ether-containing DTCs and bisphosphines have a significant impact on the lipophilicity of their corresponding cationic ^{99m}Tc-nitrido complexes. For example, [^{99m}TcN(L)(PNP6)]$^+$ is much more lipophilic than [^{99m}TcN(L)(PNP5)]$^+$ because of the high lipophilicity of the four ethoxy groups in PNP6[31]. Results from biodistribution studies showed that most of the crown ether-containing cationic ^{99m}Tc-nitrido complexes had a relatively high initial heart uptake with a long myocardial retention[31]. It was also demonstrated that [^{99m}TcN(L4)(L6)]$^+$ (^{99m}TcN-15C5) had the heart/liver ratios that were 4-5 times better than that of ^{99m}Tc-Sestamibi due to its faster liver clearance. The

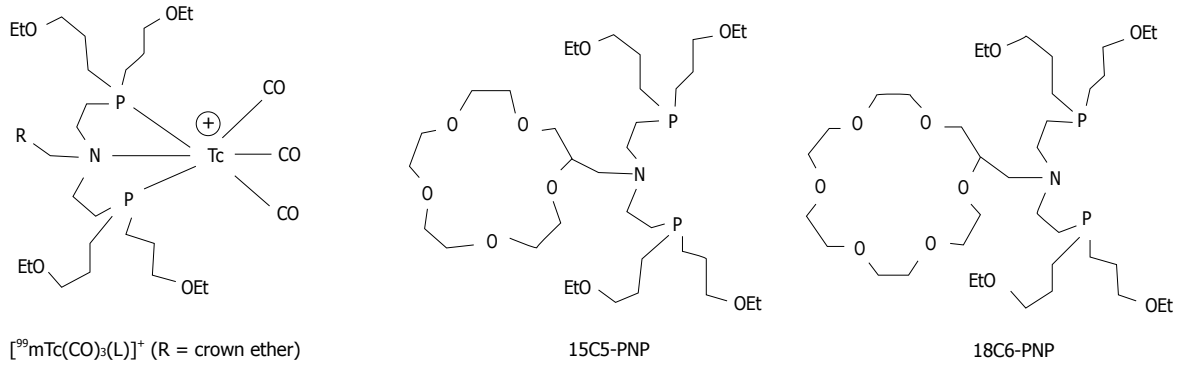

Figure 4 Cationic ^{99m}Tc(I)-tricarbonyl complexes with crown ether-containing PNP bisphosphines.

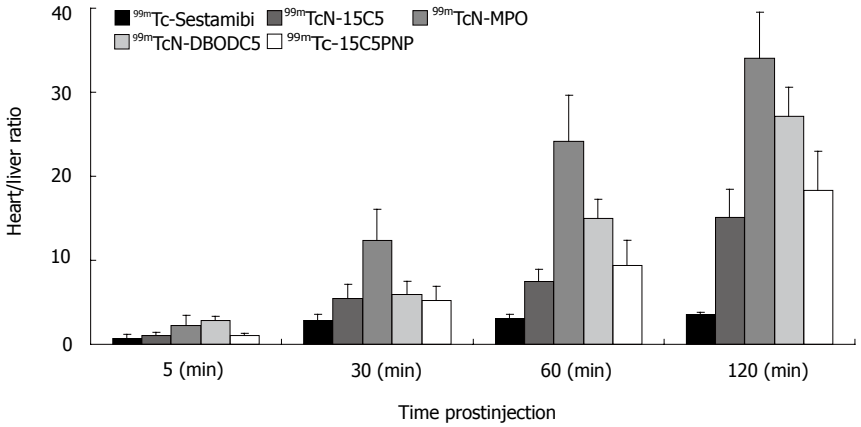

Figure 5 Comparison of heart/liver ratios between [^{99m}TcN(L4)(L6)]$^+$ (^{99m}TcN-15C5), [^{99m}TcN(mpo)(PNP5)]$^+$ (^{99m}TcN-MPO), [^{99m}Tc(CO)$_3$(15CPNP)]$^+$ (^{99m}Tc-15C5PNP), ^{99m}Tc-Sestamibi and ^{99m}TcN-DBODC5 in SD rats.

heart uptake and heart/liver ratios of ^{99m}TcN-15C5 are comparable to that of ^{99m}TcN-DBODC5[32]. The results from planar imaging and SPECT studies in dogs further confirm its faster liver clearance kinetics as compared to that of ^{99m}Tc-Sestamibi[33]. In dogs with acute myocardial infarction, ^{99m}TcN-15C5 was able to detect the perfusion defect as early as 15-30 min.

Crown ether-containing cationic ^{99m}Tc(I)-tricarbonyl complexes

The rich and diverse coordination chemistry of [^{99m}Tc(CO)$_3$]$^+$ offers a tremendous opportunity to develop new ^{99m}Tc(I)-tricarbonyl radiotracers[34-43]. The [^{99m}Tc(CO)$_3$]$^+$ core has also been widely used to prepare the target-specific ^{99m}Tc radiotracers[37-40]. In [^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$, all three water molecules are labile with respect to substitution[40,42]. Monodentate ligands, 2-methoxy-isobutylisonitriles (MIBI) and dimethyl-3-methoxypropylphosphine (DMMPP), have been used to prepare complexes [^{99m}Tc(L)$_3$(CO)$_3$]$^+$ (L = DMMPP and MIBI)[22]. In Sprague-Dawley rats, [^{99m}Tc(DMMPP)$_3$(CO)$_3$]$^+$ and [^{99m}Tc(MIBI)$_3$(CO)$_3$]$^+$ showed high heart uptake. However, their heart/liver and heart/lung ratios are not as good as that of ^{99m}Tc-Sestamibi due to slow hepatobiliary excretion[22]. The major challenge is to maintain the "true" cationic nature of [^{99m}Tc(L)$_3$(CO)$_3$]$^+$ (L = DMMPP and MIBI) in the blood circulation.

It has been reported that bidentate ligands form ^{99m}Tc(I)-tricarbonyl complexes with low solution stability, which may result in high protein binding and high background activity in blood[37,40]. The chloride in blood may react with [^{99m}Tc(L-L)(CO)$_3$]$^+$ to form neutral complexes [^{99m}Tc(L-L)(CO)$_3$Cl] that has low heart uptake and slow hepatobiliary excretion. In contrast, the PNP bisphosphines are able to form highly stable cationic complexes [^{99m}Tc(CO)$_3$(L)]$^+$ (Figure 4: L = 15C5-PNP and 18C6-PNP)[44,45]. The tridentate bisphosphines are critical to maintain the cationic nature of ^{99m}Tc(I)-tricarbonyl complexes. Among the radiotracers evaluated in SD rats, [^{99m}Tc(CO)$_3$(15C5-PNP)]$^+$ (^{99m}Tc-15C5-PNP) had a high initial heart uptake with a long myocardial retention. It also showed a rapid clearance from liver and lungs. The heart/liver ratio of ^{99m}Tc-15C5-PNP is ~2.5× better than that of ^{99m}Tc-Sestamibi at 30 min post-injection. ^{99m}Tc-15C5-PNP is almost identical to ^{99m}TcN-DBODC5 with respect to their heart uptake and heart/liver ratios[45]. Planar imaging studies also demonstrated that ^{99m}Tc-15C5-PNP had a much better liver clearance profile than ^{99m}Tc-sestamibi.

COMPARISON OF BIODISTRIBUTION CHARACTERISTICS

Figure 5 compares their heart/liver ratios in SD rats at 5, 30, 60 and 120 min post-injection. In general, the heart uptake of ^{99m}TcN-15C5, ^{99m}TcN-MPO, ^{99m}Tc-15C5PNP and ^{99m}TcN-DBODC5 are comparable within

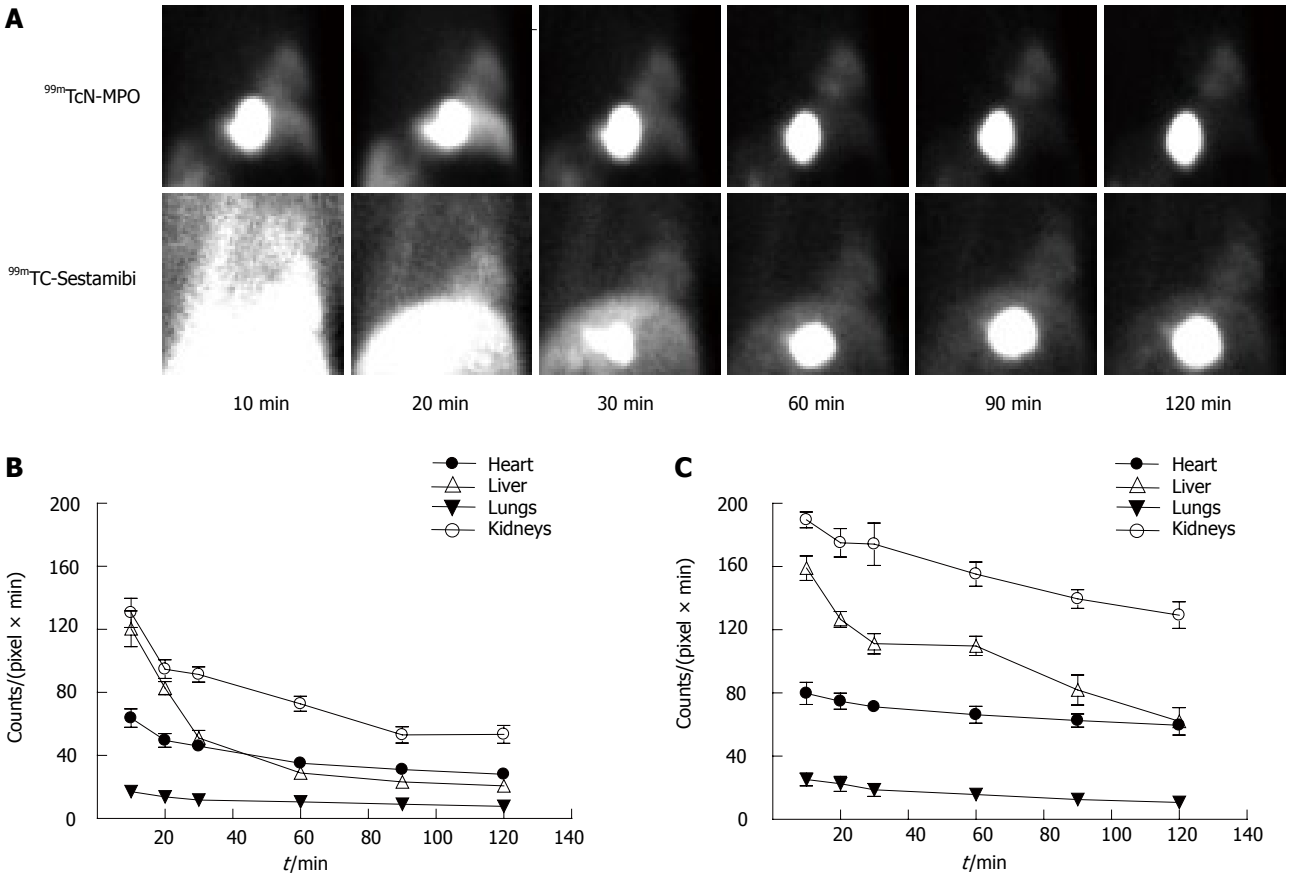

Figure 6 Planar images and organ clearance kinetics of normal dogs. A: Planar images of normal dogs administered with ^{99m}TcN-MPO and ^{99m}Tc-Sestamibi. Both radiotracers had similar initial myocardial uptake; **B: Imaging quantification in normal dogs administered with ^{99m}TcN-MPO; C: Imaging quantification in normal dogs administered with ^{99m}Tc-Sestamibi.** The liver radioactivity of ^{99m}TcN-MPO was markedly decreased within first 60 min whereas ^{99m}Tc-Sestamibi had a slower reduction in liver radioactivity over time. A mild myocardial washout was observed in the dogs administered with ^{99m}TcN-MPO. No significant myocardial washout was seen in dogs administered with ^{99m}Tc-Sestamibi.

the experimental error, and is slightly lower than that of ^{99m}Tc-Sestamibi, particularly at > 60 min post-injection. However, their heart/liver ratios are much better than that of ^{99m}Tc-Sestamibi at 30-120 min post-injection[46]. For example, the heart/liver ratio of ^{99m}TcN-MPO (12.75 ± 3.34) at 30 min post-injection is almost twice of that of ^{99m}TcN-DBODC5 (6.01 ± 1.45), and is ~4 times better than that of ^{99m}Tc-sestamibi (2.90 ± 0.22).

^{99m}TcN-MPO was further evaluated in normal dogs in comparison with ^{99m}Tc-sestamibi. It was found that ^{99m}TcN-MPO and ^{99m}Tc-sestamibi shared very similar blood clearance with < 50% of initial radioactivity remaining at 1 min, < 5% of initial radioactivity remaining at 30 min post-injection. The liver uptake in the dogs administered with ^{99m}TcN-MPO decreased rapidly whereas a prolonged liver uptake was seen in all images of the dogs administered with ^{99m}Tc-Sestamibi (Figure 6). The heart/liver ratio of ^{99m}TcN-MPO increased with time (0.53 ± 0.06 at 10 min and 1.22 ± 0.06 at 60 min post-injection), whereas the heart/liver ratio of ^{99m}Tc-Sestamibi remained relatively unchanged over the 2 h study period (0.50 ± 0.03 at 10 min and 0.60 ± 0.02 at 60 min post-injection). SPECT studies (Figure 7) in canines with acute myocardial infarction indicated that the perfusion defect could be visualized as early as 30 min after administration of ^{99m}TcN-MPO but not ^{99m}Tc-Sestamibi. The combination of fast blood clearance, relatively high heart uptake with prolonged myocardial retention makes ^{99m}TcN-MPO a better perfusion imaging radiotracer than ^{99m}Tc-Sestamibi. On the basis of preliminary results from the rats and dogs, ^{99m}TcN-MPO was selected as a clinical candidate for human studies.

Safety parameters measured up to 24 h after injection of ^{99m}TcN-MPO revealed no adverse reactions and clinically significant drug-related changes in healthy volunteers (unpublished data). The radiation dosimetry of ^{99m}TcN-MPO is comparable to that of ^{99m}Tc-Sestamibi[16] and ^{99m}Tc-Tetrofosmin[17]. Figure 8 shows the whole-body images of a healthy volunteer administered with ^{99m}TcN-MPO (~25 mCi) at 10, 30, 60 and 240 min post-injection. The radioactivity clearance was so fast that the heart was well separated from the left liver lobe at 10 min post-injection. Although its first-pass extraction fraction was between that of ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin[5,6], its fast liver clearance of ^{99m}TcN-MPO will allow early visualization of the heart in patients with CAD. The rapid liver clearance may shorten imaging protocols and permit a more precise determination of perfusion defects in the

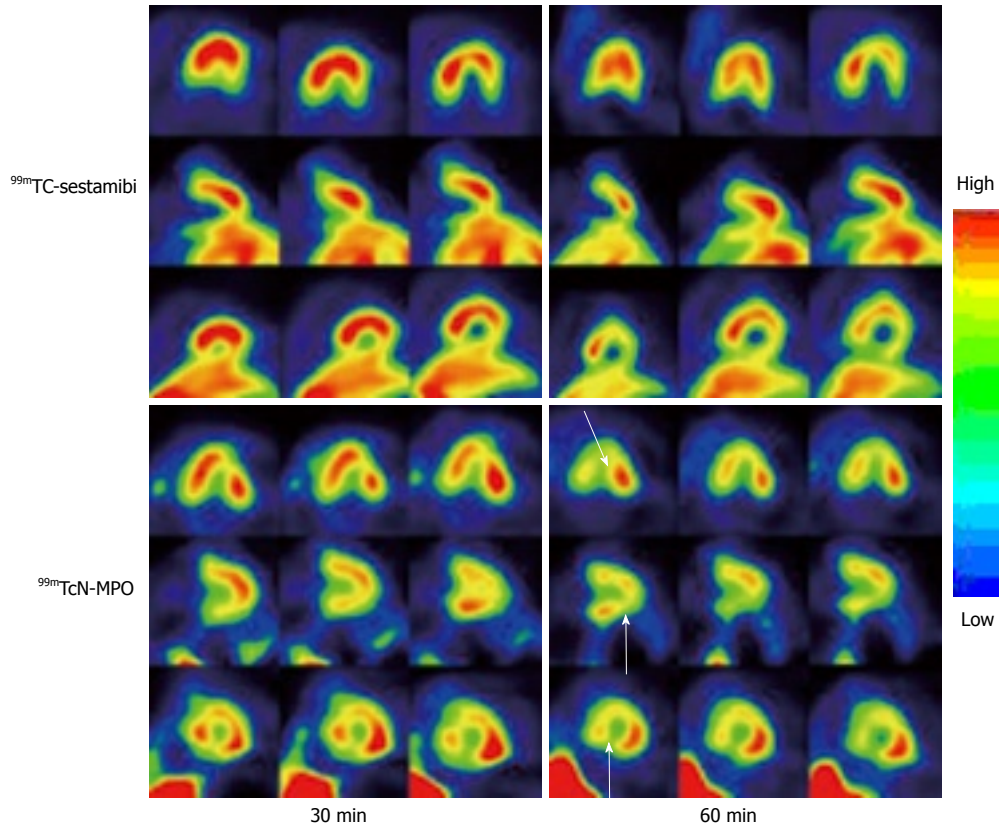

Figure 7 SPECT images of the same dog (with acute myocardial infarction) administered with ~10 mCi of ^{99m}Tc-Sestamibi and ^{99m}TcN-MPO at 30 and 60 min p.i. Arrows indicate the presence of perfusion defects due to myocardial infarction. For ^{99m}TcN-MPO, the perfusion defects were clearly seen as early as 30 min post-injection due to its fast liver clearance. For ^{99m}Tc-Sestamibi, there was a significant between the perfusion defects and the liver radioactivity.

inferoapical wall on myocardial images. ^{99m}TcN-MPO is an excellent alternative to ^{99m}Tc-Sestamibi for myocardial perfusion imaging when the liver uptake makes it difficult to interpret the heart activity in the inferior and left ventricular wall in patients with known or suspected CAD.

LOCALIZATION MECHANISM

Optimal log P values

Myocardium has the highest mitochondrial population that occupies up to 40% of the total volume of myocytes. Other mitochondria-rich organs include salivary glands, liver and kidneys. This may explain why most lipophilic ^{99m}Tc complex cations tend to have high uptake in mitochondria-rich organs, such as heart, liver and kidneys. For a ^{99m}Tc radiotracer to localize in mitochondria, it must be able to across plasma and mitochondrial membrane. Regardless of their charge, most small molecules are able to across the plasma membrane without significant difficulty. However, the mitochondrial membrane is only permeable to those molecules with appropriate molecular charge and molecular shape. While the contribution from mitochondrial potentials provides a driving force for mitochondrial localization of cationic ^{99m}Tc radiotracers, the lipophilicity might modulate their penetration capability across the lipophilic plasma and mitochondrial membranes. There is little information available with regard to the optimal lipophilicity for the heart-selectivity. Cationic ^{99m}Tc radiotracers with the log *P* values > 1.5 often shows a high protein binding and a slow clearance from non-cardiac organs while hydrophilic cationic radiotracers with log *P* < 0.5 usually show a low heart uptake with a fast washout from myocardium[30-32,45]. In both cases, the heart/liver ratio is low because of either high liver uptake or fast myocardial washout. On the basis of studies on cationic ^{99m}Tc-nitrido and ^{99m}Tc(I)-tricarbonyl complexes, it seems that cationic ^{99m}Tc radiotracers must have a log *P* value in the range of 0.9-1.2 in order to achieve a high heart uptake[30-32,45,46].

Subcellular distribution characteristics and mitochondrial localization

To understand the myocardial localization mechanism, the subcellular distribution characteristics of ^{99m}TcN-MPO[47] and ^{99m}TcN-DBODC5[48] in SD rat hearts were analyzed in comparison with ^{99m}Tc-Sestamibi according to the literature[49,50]. It was clearly demonstrated that more than 85% of myocardial radioactivity localized in the mitochondria of myocytes for both ^{99m}TcN-MPO and ^{99m}TcN-DBODC5. There was no significant difference between ^{99m}TcN-MPO, ^{99m}TcN-DBODC5 and ^{99m}Tc-Sestamibi with respect to their mitochondrial radioactivity accumulation, and subcellular distribution characteristics.

MULTIDRUG RESISTANCE GENE EXPRESSION AND LIVER CLEARANCE KINETICS

For the last two decades, many cationic ^{99m}Tc radiotracers have been evaluated for their potential as radiotracers

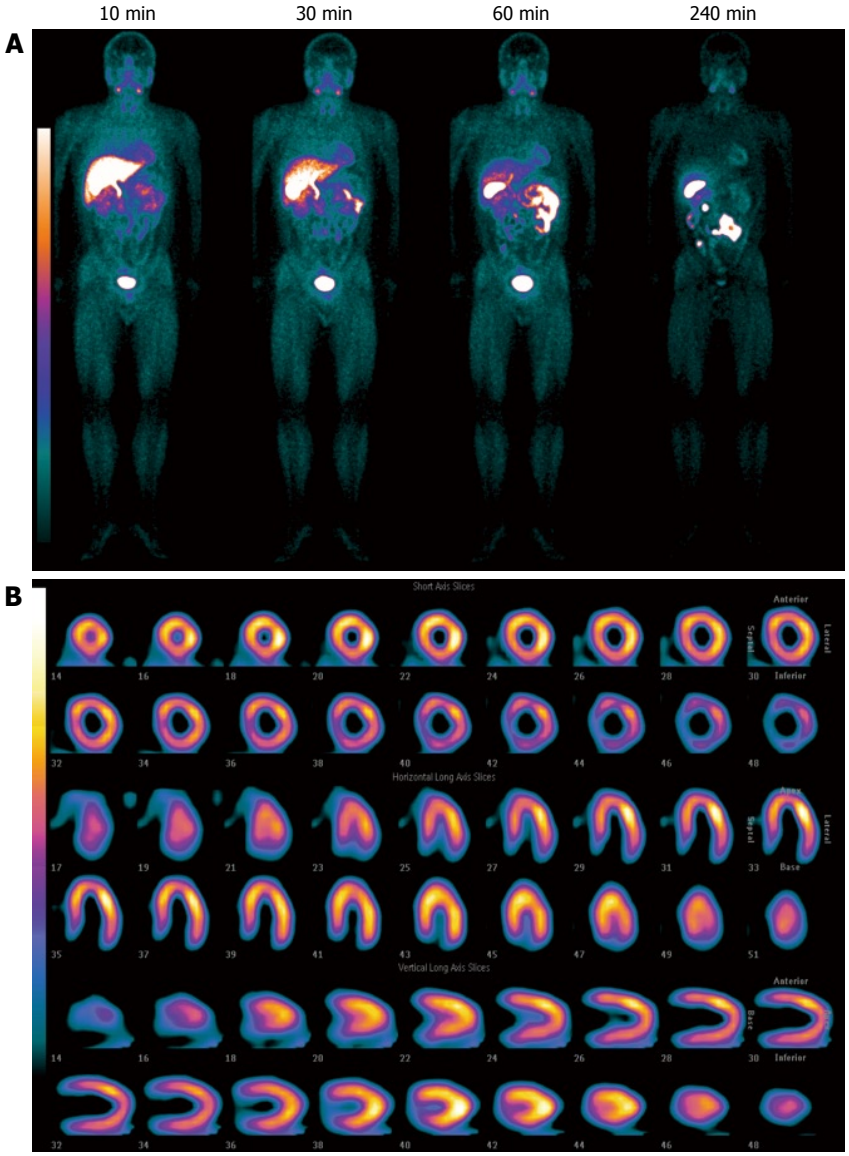

Figure 8 The whole-body images of representative a healthy volunteer administered with ^{99m}TcN-MPO. A: Administered with ^{99m}TcN-MPO (~25 mCi) at 10, 30, 60 and 240 min post-injection; B: Representative SPECT images of the heart **after administration of** ^{99m}TcN-MPO (~25 mCi) at 60 min post-injection.

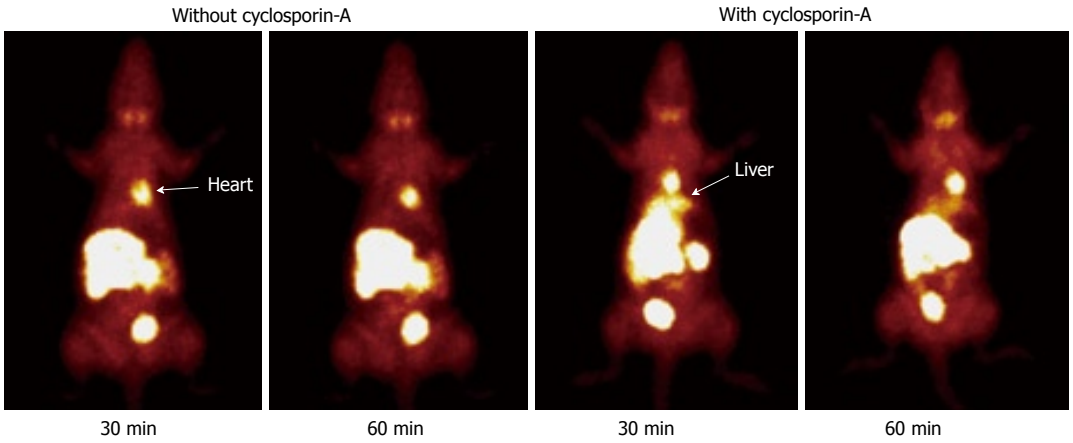

Figure 9 Planar images of the SD rats administered with ~1.0 mCi of ^{99m}TcN-MPO in the absence and presence of cyclosporin-A at 30 and 60 min post-injection. Pre-treatment with cyclosporin-A (14 mg/kg) results in more liver radioactivity accumulation and slower liver clearance.

for heart imaging. However, it is not clear why certain cationic ^{99m}Tc radiotracers show better liver clearance and heart/liver ratios than others, even though they may have a similar lipophilicity. It is well-documented that cationic ^{99m}Tc radiotracers, such as ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin, are also clinically useful for noninvasive imaging of multidrug resistance (*MDR*) in tumors of different origin[51-53,57,58]. It is well-documented that MDR1 P-glycoprotein (MDR1 Pgp) and multidrug resistance associated proteins (MRPs) are overexpressed in normal organs that are involved in excretory functions, including kidneys and liver[54-56]. For example, an increased ^{99m}Tc-

Sestamibi uptake in normal liver of cancer patients has been reported after administration of P-glycoprotein inhibitors[59,60]. To demonstrate the correlation between the MDR and MRP transport function of hepatocytes and liver excretion kinetics of ^{99m}TcN-MPO, both biodistribution and imaging studies were performed using the SD rats in the absence/presence of Cyclosporin A. It was found that the uptake of ^{99m}TcN-MPO in the kidneys and liver was significantly increased, and the radioactivity excretion was delayed, in the presence of Cyclosporin A. Similar results were also obtained in planar images (Figure 9) of SD rats administered with ^{99m}TcN-MPO with/without excess Cyclosporin A. Thus, the MDR/MRP transport function is most likely responsible of the fast efflux of ^{99m}TcN-MPO from kidneys and liver.

CONCLUSION

SPECT remains the modality of choice for myocardial perfusion imaging in current clinical practice. The success of SPECT in nuclear cardiology is largely due to the development of new ^{99m}Tc perfusion radiotracers. Studies on ether- and crown ether-containing cationic ^{99m}Tc complexes clearly show that it is possible to design cationic ^{99m}Tc radiotracers with heart/liver ratios substantially better than that of ^{99m}Tc-Sestamibi. Fast liver radioactivity clearance will shorten the duration of imaging protocols (< 30 min post-injection), and allow for early acquisition of images of high quality. Improvement of the heart/liver ratio will permit better detection of perfusion defects with improved noninvasive assessment of the myocardial perfusion. Preliminary studies on ^{99m}TcN-MPO show that it may have a lower first-pass extraction fraction than ^{99m}Tc-Sestamibi. Thus, future research should focus on cationic ^{99m}Tc perfusion radiotracers with both fast liver clearance and better first-pass extraction fraction. Identification of such a new radiotracer would be of considerable benefit in treatment of patients with suspected CAD.

REFERENCES

1 **Nunn AD**. Radiopharmaceuticals for imaging myocardial perfusion. *Semin Nucl Med* 1990; **20**: 111-118
2 **Saha GB**, Go RT, MacIntyre WJ. Radiopharmaceucticals for cardiovascular imaging. *Int J Rad Appl Instrum B* 1992; **19**: 1-20
3 **Opie LH, Hesse B**. Radionuclide tracers in the evaluation of resting myocardial ischaemia and viability. *Eur J Nucl Med* 1997; **24**: 1183-1193
4 **Berman DS**, Germano G, Shaw LJ. The role of nuclear cardiology in clinical decision making. *Semin Nucl Med* 1999; **29**: 280-297
5 **Jain D**. Technetium-99m labeled myocardial perfusion imaging agents. *Semin Nucl Med* 1999; **29**: 221-236
6 **Beller GA**, Bergmann SR. Myocardial perfusion imaging agents: SPECT and PET. *J Nucl Cardiol* 2004; **11**: 71-86
7 **Acampa W**, Di Benedetto C, Cuocolo A. An overview of radiotracers in nuclear cardiology. *J Nucl Cardiol* 2000; **7**: 701-707
8 **Dilsizian V**. The role of myocardial perfusion imaging in vascular endothelial dysfunction. *J Nucl Cardiol* 2000; **7**: 180-184
9 **Beller GA**, Zaret BL. Contributions of nuclear cardiology to diagnosis and prognosis of patients with coronary artery disease. *Circulation* 2000; **101**: 1465-1478
10 **Parker JA**. Cardiac nuclear medicine in monitoring patients with coronary heart disease. *Semin Nucl Med* 2001; **31**: 223-237
11 **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. A comparison of three radionuclide myocardial perfusion tracers in clinical practice: the ROBUST study. *Eur J Nucl Med Mol Imaging* 2002; **29**: 1608-1616
12 **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**: 482-498
13 **Banerjee S**, Pillai MR, Ramamoorthy N. Evolution of Tc-99m in diagnostic radiopharmaceuticals. *Semin Nucl Med* 2001; **31**: 260-277
14 **Llaurado JG**. The quest for the perfect myocardial perfusion indicator still a long way to go. *J Nucl Med* 2001; **42**: 282-284
15 **Jones AG**, Abrams MJ, Davison A, Brodack JW, Toothaker AK, Adelstein SJ, Kassis AI. Biological studies of a new class of technetium complexes: the hexakis(alkylisonitrile)technetium(I) cations. *Int J Nucl Med Biol* 1984; **11**: 225-234
16 **Wackers FJ**, Berman DS, Maddahi J, Watson DD, Beller GA, Strauss HW, Boucher CA, Picard M, Holman BL, Fridrich R. Technetium-99m hexakis 2-methoxyisobutyl isonitrile: human biodistribution, dosimetry, safety, and preliminary comparison to thallium-201 for myocardial perfusion imaging. *J Nucl Med* 1989; **30**: 301-311
17 **Higley B**, Smith FW, Smith T, Gemmell HG, Das Gupta P, Gvozdanovic DV, Graham D, Hinge D, Davidson J, Lahiri A. Technetium-99m-1,2-bis[bis(2-ethoxyethyl) phosphino]ethane: human biodistribution, dosimetry and safety of a new myocardial perfusion imaging agent. *J Nucl Med* 1993; **34**: 30-38
18 **Iskandrian AS**, Heo J, Kong B, Lyons E, Marsch S. Use of technetium-99m isonitrile (RP-30A) in assessing left ventricular perfusion and function at rest and during exercise in coronary artery disease, and comparison with coronary arteriography and exercise thallium-201 SPECT imaging. *Am J Cardiol* 1989; **64**: 270-275
19 **Marmion ME**, Woulfe SR, Neumann WL, Nosco DL, Deutsch E. Preparation and characterization of technetium complexes with Schiff base and phosphine coordination. 1. Complexes of technetium-99g and -99m with substituted acac$_2$en and trialkyl phosphines (where acac$_2$en = N,N'-ethylenebis[acetylacetone iminato]). *Nucl Med Biol* 1999; **26**: 755-770
20 **Lisic EC**, Heeg MJ, Deutsch E. ^{99m}Tc(L-L)$^{3+}$ complexes containing ether analogs of DMPE. *Nucl Med Biol* 1999; **26**: 563-571
21 **Tisato F**, Maina T, Shao LR, Heeg MJ, Deutsch E. Cationic [^{99m}TcIII(DIARS)$_2$(SR)$_2$]$^+$ complexes as potential myocardial perfusion imaging agents (DIARS = o-phenylenebis(dimethylarsine);SR- = thiolate). *J Med Chem* 1996; **39**: 1253-1261
22 **Marmion ME**, MacDonald JR. Preparation and biodistribution of ^{99m}Tc(I)-tricarbonyl complexes containing isonitrile and phosphine ligands. *J Nucl Med* 2000; **41**: 124P (abstract No. 489)
23 **Maresca KP**, Keith D, Graham-Coco WA, Banerjee SR, Zubieta JA, Babich JW. Novel ether containing ligand complexes of ^{99m}Tc(CO)$^{3+}$ for cardiac imaging. *J Nucl Med* 2004; **45**: 215P (abstract No. 703)
24 **Bolzati C**, Boschi A, Uccelli L, Tisato F, Refosco F, Cagnolini A, Duatti A, Prakash S, Bandoli G, Vittadini A. Chemistry of the strong electrophilic metal fragment [(99)Tc(N)(PXP)]$^{(2+)}$ (PXP

= diphosphine ligand). A novel tool for the selective labeling of small molecules. *J Am Chem* Soc 2002; **124**: 11468-11479
25 **Bolzati C**, Refosco F, Cagnolini A, Tisato F, Boschi A, Duatti A, Uccelli L, Dolmella A, Marotta E, Tubaro M. Synthesis, solution-state and solid-state structural characterization of monocationic nitrido heterocomplexes [M(N) (DTC)(PNP)]$^+$ (M = ^{99}Tc and Re; DTC = dithiocarbamate; PNP = heterodiphosphane). *Eur J Inorg Chem* 2004; 1902-1913
26 **Boschi A**, Bolzati C, Uccelli L, Duatti A, Benini E, Refosco F, Tisato F, Piffanelli A. A class of asymmetrical nitrido ^{99m}Tc heterocomplexes as heart imaging agents with improved biological properties. *Nucl Med Commun* 2002; **23**: 689-693
27 **Boschi A**, Uccelli L, Bolzati C, Duatti A, Sabba N, Moretti E, Di Domenico G, Zavattini G, Refosco F, Giganti M. Synthesis and biologic evaluation of monocationic asymmetric ^{99m}Tc-nitride heterocomplexes showing high heart uptake and improved imaging properties. *J Nucl Med* 2003; **44**: 806-814
28 **Hatada K**, Riou LM, Ruiz M, Yamamichi Y, Duatti A, Lima RL, Goode AR, Watson DD, Beller GA, Glover DK. ^{99m}Tc-N-DBODC5, a new myocardial perfusion imagingagent with rapid liver clearance: comparison with ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin in rats. *J Nucl Med* 2004; **45**: 2095-2101
29 **Hatada K**, Ruiz M, Riou LM, Lima RL, Goode AR, Watson DD, Beller GA, Glover DK. 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**: 779-790
30 **Liu S**. Ether and crown ether-containing cationic ^{99m}Tc complexes useful as radiopharmaceuticals for heart imaging. *Dalton Trans* 2007; 1183-1193
31 **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**: 929-936
32 **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**: 419-432
33 **Fang W**, Liu Y, Zhu L, Kim YS, Liu S, He ZX. Evaluation of ^{99m}TcN-15C5 as a new myocardial perfusion imaging agent in normal dogs and canines with coronary stenosis. *Nucl Med Commun* 2008; **29**: 775-781
34 **Alberto R**, Schibli R, Egli A, Schubiger PA, Abram U, Kaden TA. A novel organometallic aqua complex of technetium for the labeling of biomolecules: synthesis of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ from $[^{99m}TcO_4]^-$ in aqueous solution and its reaction with a bifunctional ligand. *J Am Chem Soc* 1998; **120**: 7987-7988
35 **Alberto R**, Ortner K, Wheatley N, Schibli R, Schubiger AP. Synthesis and properties of boranocarbonate: a convenient in situ CO source for the aqueous preparation of $[^{(99m)}Tc(OH_{(2)})_3(CO)_3]^+$. *J Am Chem Soc* 2001; **123**: 3135-3136
36 **Alberto R**, Schibli R, Abram U, Egli A, Knapp FF, Schubiger PA. Potential of the "$[M(CO)_3]^+$" (M = Re, Tc) moiety for the labeling of biomolecules. *Radiochim. Acta* 1997; **79**: 99-103
37 **Alberto R**, Schibli R, Waibel R, Abram U, Schubiger PA. Basic aqueous chemistry of $[M(H_2O)_3(CO)_3]^+$ (M = Re, Tc) directed towards radiopharmaceutical application. *Coord Chem Rev* 1999; **190-192**: 901-919
38 **Schubiger PA**, Grünberg J, Ametamey SM, Honer M, Garcia-Garayoa E, Bläuenstein P, Waibel R, Novak-Hofer I, Schibli R. Radiopharmaceuticals: from molecular imaging to targeted radionuclide therapy. *Chimia* 2004; **58**: 731-735
39 **Banerjee SR**, Maresca KP, Francesconi L, Valliant J, Babich JW, Zubieta J. New directions in the coordination chemistry of ^{99m}Tc: a reflection on technetium core structures and a strategy for new chelate design. *Nucl Med Biol* 2005; **32**: 1-20
40 **Schibli R**, Schubiger PA. Current use and future potential of organometallic radiopharmaceuticals. *Eur J Nucl Med Mol Imaging* 2002; **29**: 1529-1542
41 **Häfliger P**, Mundwiler S, Ortner K, Spingler B, Alberto R, Andócs G, Balogh L, Bodo K. Structure, stability, and biodistribution of cationic $[M(CO)_3]^+$ (M = Re, ^{99}Tc, ^{99m}Tc) complexes with tridentate amine ligands. *Synth React Inorg Met-Org Chem* 2005; **35**: 27-34
42 **Rattat D**, Eraets K, Cleynhens B, Knight H, Fonge H, Verbruggen A. Comparison of tridentate ligands in competition experiments for their ability to form a $[^{99m}Tc(CO)_3]^+$ complex. *Tetrahedron Lett* 2004; **45**: 2531-2534
43 **Schibli R**, La Bella R, Alberto R, Garcia-Garayoa E, Ortner K, Abram U, Schubiger PA. Influence of the denticity of ligand systems on the in vitro and in vivo behavior of (99m)Tc(I)-tricarbonyl complexes: a hint for the future functionalization of biomolecules. *Bioconjug Chem* 2000; **11**: 345-351
44 **Kim YS**, He Z, Schibli R, Liu S. Synthesis, characterization and X-ray crystal structure of $[Re(PNP)(CO)_3]Br \cdot 2CH_3OH$: a model compound for a novel cationic ^{99m}Tc radiotracers useful for heart imaging. *Inorg Chim Acta* 2006; **359**: 2479-2488
45 **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**: 1045-1053
46 **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**: 535-546
47 **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**: 571-579
48 **Bolzati C**, Cavazza-Ceccato M, Agostini S, Tokunaga S, Casara D, Bandoli G. Subcellular distribution and metabolism studies of the potential myocardial imaging agent $[^{99m}Tc(N)(DBODC)(PNP_5)]^+$. *J Nucl Med* 2008; **49**: 1336-1344
49 **Carvalho PA**, Chiu ML, Kronauge JF, Kawamura M, Jones AG, Holman BL, Piwnica-Worms D. Subcellular distribution and analysis of technetium-99m-MIBI in isolated perfused rat hearts. *J Nucl Med* 1992; **33**: 1516-1522
50 **Piwnica-Worms D**, Kronauge JF, Chiu ML. Uptake and retention of hexakis (2-methoxyisobutyl isonitrile) technetium(I) in cultured chick myocardial cells. *Mitochondrial and plasma membrane potential dependence. Circulation* 1990; **82**: 1826-1838
51 **Sharma V**, Piwnica-Worms D. Metal complexes for therapy and diagnosis of drug resistance. *Chem Rev* 1999; **99**: 2545-2560
52 **Sharma V**. Radiopharmaceuticals for assessment of multidrug resistance P-glycoprotein-mediated drug transport activity. *Bioconjug Chem* 2004; **15**: 1464-1474
53 **Vaidyanathan G**, Zalutsky MR. Imaging drug resistance with radiolabeled molecules. *Curr Pharm Des* 2004; **10**: 2965-2979
54 **Gatmaitan ZC**, Arias IM. Structure and function of P-glycoprotein in normal liver and small intestine. *Adv Pharmacol* 1993; **24**: 77-97
55 **Lee CH**, Bradley G, Zhang JT, Ling V. Differential expression of P-glycoprotein genes in primary rat hepatocyte culture. *J Cell Physiol* 1993; **157**: 392-402
56 **Mayer R**, Kartenbeck J, Büchler M, Jedlitschky G, Leier I, Keppler D. Expression of the MRP gene-encoded conjugate export pump in liver and its selective absence from the canalicular membrane in transport-deficient mutant hepatocytes. *J Cell Biol* 1995; **131**: 137-150
57 **Dyszlewski M**, Blake HM, Dahlheimer JL, Pica CM, Piwnica-Worms D. Characterization of a novel ^{99m}Tc-carbonyl complex as a functional probe of MDR1 P-glycoprotein transport activity. *Mol Imaging* 2002; **1**: 24-35
58 **Muzzammil T**, Ballinger JR, Moore MJ. ^{99}Tcm-sestamibi imaging of inhibition of the multidrug resistance transporter in a mouse xenograft model of human breast cancer. *Nucl Med Commun* 1999; **20**: 115-122
59 **Luker GD**, Fracasso PM, Dobkin J, Piwnica-Worms D. Mo-

dulation of the multidrug resistance P-glycoprotein: detection with technetium-99m-sestamibi in vivo. *J Nucl Med* 1997; **38**: 369-372

60 **Agrawal M**, Abraham J, Balis FM, Edgerly M, Stein WD, Bates S, Fojo T, Chen CC. Increased ^{99m}Tc-sestamibi accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor, XR9576. *Clin Cancer Res* 2003; **9**: 650-656

S- Editor Zhang HN **L- Editor** Negro F **E- Editor** Liu N