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Effect of co-ligands on chemical and biological properties of ^{99m}Tc(III) complexes [^{99m}Tc(L)(CDO)(CDOH)$_2$BMe] (L = Cl, F, SCN and N_3; $CDOH_2$ = cyclohexanedione dioxime)

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ABSTRACT

Introduction: ^{99m}Tc-Teboroxime ([^{99m}TcCl(CDO)(CDOH)$_2$BMe]) is a member of the BATO (boronic acid adducts of technetium dioximes) class of ^{99m}Tc(III) complexes. This study sought to explore the impact of co-ligands on solution stability, heart uptake and myocardial retention of [^{99m}Tc(L)(CDO)(CDOH)$_2$BMe] (^{99m}Tc-Teboroxime: L = Cl; ^{99m}Tc-Teboroxime(F): L = F; ^{99m}Tc-Teboroxime(SCN): L = SCN; and ^{99m}Tc-Teboroxime(N_3): L = N_3).
Methods: Radiotracers ^{99m}Tc-Teboroxime(L) (L = F, SCN and N_3) were prepared by reacting ^{99m}Tc-Teboroxime with NaF, NaSCN and NaN_3, respectively. Biodistribution and imaging studies were carried out in Sprague–Dawley rats. Image quantification was performed to compare their heart retention and liver clearance kinetics.
Results: Complexes ^{99m}Tc-Teboroxime(L) (L = F, SCN and N_3) were prepared in high yield with high radiochemical purity. All new radiotracers were stable for >6 h in the kit matrix. In its HPLC chromatogram, ^{99m}Tc-Teboroxime showed one peak at ~15.5 min, which was shorter than that of ^{99m}Tc-Teboroxime(F) (~16.4 min). There were two peaks for ^{99m}Tc-Teboroxime(SCN) at 16.5 and 18.3 min. ^{99m}Tc-Teboroxime(N_3) appeared as a single peak at 18.4 min. Their heart retention and liver clearance curves were best fitted to the bi-exponential decay function. The half-times of fast/slow components were 1.6 ± 0.4/60.7 ± 8.9 min for ^{99m}Tc-Teboroxime, 0.8 ± 0.2/101.7 ±20.7 min for ^{99m}Tc-Teboroxime(F), 1.2 ± 0.3/84.8 ± 16.6 min for ^{99m}Tc-Teboroxime(SCN), and 2.9 ± 0.9/51.6 ±5.0 min for ^{99m}Tc-Teboroxime(N_3). The 2-min heart uptake followed the order of ^{99m}Tc-Teboroxime (3.00 ± 0.37%ID/g) > ^{99m}Tc-Teboroxime(N_3) (2.66 ± 0.01 %ID/g) ≈ ^{99m}Tc-Sestamibi (2.55 ± 0.46 %ID/g) > ^{99m}TcN-MPO (2.38 ± 0.15 %ID/g). ^{99m}Tc-Teboroxime remains the best in first-pass extraction. The best image acquisition window is 0–5 min for ^{99m}Tc-Teboroximine and 0–15 min for ^{99m}Tc-Teboroximine(N_3).
Conclusion: Co-ligands had significant impact on the heart uptake and myocardial retention of complexes [^{99m}Tc(L)(CDO)(CDOH)$_2$BMe] (L = Cl, F, SCN and N_3). Future studies should be directed towards minimizing the liver uptake and radioactivity accumulation in the blood vessels while maintaining their high heart uptake.

1. Introduction

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

A significant drawback of ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin is their low first-pass extraction [15–18]. In contrast, ^{99m}Tc-Teboroxime (Fig. 1: [^{99m}TcCl(CDO)(CDOH)$_2$BMe]; $CDOH_2$ = cyclohexanedione dioxime), is a member of the BATO (boronic acid adducts of technetium

Abbreviations: BATO, boronic acid adducts of technetium dioximes; CAD, coronary artery disease; DTPA, diethylenetriaminepentaacetic acid (or pentetic acid); MPI, myocardial perfusion imaging; RCP, radiochemical purity; SPECT, single photon-emission computed tomography; ^{99m}TcN-MPO, [^{99m}TcN(mpo)(PNP5)]$^+$ (mpo = 2-mercaptopyridine oxide, and PNP5 = N-ethoxyethyl-N,N-bis[2-(bis(3-methoxypropyl)phosphino)ethyl]amine); ^{99m}TcN-NOET, ^{99m}TcN(NOET)$_2$ (NOET = N-ethoxy-N-ethyldithiocarbamato); ^{99m}Tc-Sestamibi, [^{99m}Tc(MIBI)$_6$]$^+$ (MIBI = 2-methoxy-2-methylpropylisonitrile); ^{99m}Tc-Tetrofosmin, [^{99m}TcO$_2$(tetrofosmin)$_2$]$^+$ (tetrofosmin = 1,2-bis[bis(2-ethoxyethyl)phosphino] ethane); ^{99m}Tc-Teboroxime, [^{99m}TcCl(CDO)(CDOH)$_2$BMe] ($CDOH_2$ = cyclohexanedione dioxime); ^{99m}Tc-Teboroxime(F), [^{99m}Tc(F)(CDO)(CDOH)$_2$BMe]; ^{99m}Tc-Teboroxime(SCN), [^{99m}Tc(SCN)(CDO)(CDOH)$_2$BMe]; ^{99m}Tc-Teboroxime(N_3), [^{99m}Tc(N_3)(CDO)(CDOH)$_2$BMe].

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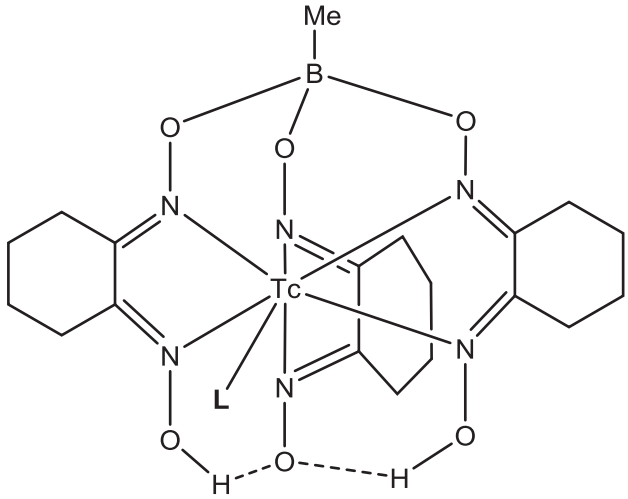

Fig. 1. Structures of ^{99m}Tc(III) radiotracers [^{99m}Tc (L)(CDO)$(CDOH)_2$BMe] (^{99m}Tc-Teboroxime: L = Cl; ^{99m}Tc-Teboroxime(F): L = F; ^{99m}Tc-Teboroxime(SCN): L = SCN; and ^{99m}Tc-Teboroxime(N_3): L = N_3). ^{99m}Tc-Teboroxime was the first FDA-approved radiotracer for MPI; but it was later withdrawn from the market due to its fast washout from heart. ^{99m}Tc-Teboroxime(F), ^{99m}Tc-Teboroxime(SCN) and ^{99m}Tc-Teboroxime(N_3) are new radiotracers evaluated in this study.

dioximes) class of ^{99m}Tc(III) complexes [19–21], has the highest first-pass extraction fraction among all ^{99m}Tc perfusion radiotracers [22–30]. There is an excellent linear relationship between its heart uptake and blood flow over a wide range (0–4.5 mL/min/g) [24,25,27,28]. High first-pass extraction and linear relationship between the blood flow and radiotracer heart uptake permit better detection of the presence and extent of coronary disease, and more precise delineation of perfusion defects, which is of considerable benefit in the management of patients with known or suspected CAD and assessing risk of future cardiac events (e.g. myocardial infarction and sudden death). Despite this advantage, its initial clinical experience was disappointing due to its short myocardial residence time. More than 60% of the initial heart radioactivity is washed out within 5 min post-injection (p.i.) [28–30], which is too fast for standard SPECT cameras to acquire high-quality images. As a result, ^{99m}Tc-Teboroxime was withdrawn from the market even though it was the first FDA-approved radiotracer for MPI. However, the recent developments in high-speed cardiac SPECT cameras make it possible to use ^{99m}Tc-Teboroxime for SPECT MPI due to its high first-pass extraction fraction [31].

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

2. Experimental methods

2.1. Materials

w?>Citric acid, γ-cyclodextrin (γ-CD), cyclohexanedione dioxime ($CDOH_2$), diethylenetriaminepentaacetic acid (DTPA), 1,2-diaminopropane-N,N,N′,N′-tetraacetic acid (PDTA), methylboronic acid, sodium chloride, sodium fluoride, sodium azide, sodium thiocyanate, and stannous chloride dihydrate ($SnCl_2{\cdot}2H_2O$) were purchased from Sigma/Aldrich (St. Louis, MO), and were used without further purification. ^{99m}TcN-MPO([^{99m}TcN(mpo)(PNP5)]$^+$: PNP5 = N-ethoxyethyl-N,N-bis[2-(bis(3-methoxypropyl)phosphino)ethyl]amine)) was prepared according to literature methods [40–44], and its radiochemical purity (RCP) was >95% before being used for biodistribution study. $Na^{99m}TcO_4$ were obtained from Cardinal HealthCare® (Chicago, IL). Cardiolite® vials were obtained as a gift from Lantheus Medical Imaging (formerly Bristol Myers Squibb Medical Imaging, N. Billerica, MA). ^{99m}Tc-Sestamibi was prepared according to the manufacturer's package insert.

2.2. Radio-HPLC method

The radio-HPLC method for routine analysis of ^{99m}Tc(III) complexes [^{99m}Tc(L)(CDO)$(CDOH)_2$BMe] (L = F, SCN and N_3) used the Agilent HP-1100 HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a β-ram IN/US detector (Tampa, FL) and Zorbax C_8 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 15 min and continued till 20 min. The instant thin layer chromatography (ITLC) used Gelman Sciences silica-gel strips and a 1:1 mixture of acetone and saline as the mobile phase. ^{99m}Tc(III) complexes and $^{99m}TcO_4^-$ 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.

2.3. ^{99m}Tc-teboroxime

^{99m}Tc-Teboroxime was prepared using the kit formulation. Each lyophilized vial contains 2 mg of $CDOH_2$, 2mg of methylboronic acid, 50 μg of $SnCl_2{\cdot}2H_2O$, 9 mg of citric acid, 2 mg of DTPA, 100 mg of NaCl and 20 mg of γ-cyclodextrin. To a lyophilized vial was added 1.0 mL $^{99m}TcO_4^-$ solution (10–30 mCi). The reconstituted vial was then heated at 100°C for 10–15 min. After radiolabeling, the vial was allowed to stand at room temperature for 5 min. A sample of the resulting solution was analyzed by radio-HPLC. The RCP was 95–98% with minimal amount of [^{99m}Tc]colloid (<0.5%).

2.4. ^{99m}Tc-teboroxime(L) (L = F, SCN and N_3)

^{99m}Tc-Teboroxime was prepared using the procedure above. After radiolabeling, part of the solution (0.3–0.5 mL) containing 5–15 mCi of ^{99m}Tc-Teboroxime was transferred into a sealed 5 mL vial, which contains sodium fluoride (2–5 mg), sodium azide (2–3 mg) or sodium thiocyanate (2–3 mg) dissolved in dissolved in 0.5 mL of