A Gallium-68 Radiopharmaceutical That is Retained in Myocardium: $^{68}Ga[(4,6\text{-}MeO_2sal)_2BAPEN]^+$

Brenda W. Tsang, Carla J. Mathias and Mark A. Green

Department of Medicinal Chemistry, School of Pharmacy, Purdue University, West Lafayette, Indiana

The cationic gallium(III) complex formed with the *bis*(4,6-dimethoxy)salicylaldimine of *N,N'-bis*(3-aminopropyl)ethylenediamine has been investigated as a potential ^{68}Ga radiopharmaceutical for imaging the heart with PET. The ^{67}Ga complex of this ligand was prepared by ligand exchange from ^{67}Ga-acetylacetonate and its biodistribution determined in ether anesthetized rats following intravenous injection. At 1 min postinjection, 1% of the injected dose was found in the heart with heart-to-blood and heart-to-lung ratios of 2.3:1 and 1.9:1, respectively. No clearance of ^{67}Ga radioactivity from the heart was observed over the 1-min to 2-hr time frame studied. The ^{68}Ga complex of this ligand was also prepared and the tracer further evaluated in a PET imaging study with a normal dog. Beyond 20 min postinjection, the heart was clearly delineated in the ^{68}Ga PET images with good heart-to-blood and heart-to-lung contrast. No clearance of myocardial ^{68}Ga radioactivity was observed over the 90-min imaging period, which is consistent with the results obtained in the rat. Gallium-68 complexes of this type may be useful as radiopharmaceuticals for imaging the heart with PET.

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The development of positron-emitting radiopharmaceuticals labeled with generator-produced radionuclides could facilitate more widespread use of positron emission tomography (PET) in clinical nuclear medicine (*1–3*). The $^{68}Ge/^{68}Ga$ parent/daughter pair is particularly attractive as a source of PET radiopharmaceuticals due to the favorable half-lives of both the parent and daughter radionuclides. The 271-day half-life of the ^{68}Ge parent gives this generator a long shelf-life, while the 68-min half-life of the ^{68}Ga daughter is long enough to allow the synthesis of a wide variety of radiopharmaceuticals. In addition, if suitable ^{68}Ga radiopharmaceuticals can be developed, the 68-min half-life is attractive because it could allow long image acquisition periods as well as radiopharmaceutical administration at a site remote from the PET camera.

An important clinical application of PET is in the determination of regional myocardial perfusion. Several potential ^{68}Ga radiopharmaceuticals for myocardial perfusion imaging have previously been described (*4–7*), but none have progressed to human clinical trials. The neutral $^{68}Ga[(5\text{-}MeOsal)_3tame]$ and $^{68}Ga[(sal)_3tame\text{-}O\text{-}iso\text{-}Bu]$ complexes and the cationic $^{68}Ga[BAT\text{-}TECH]^+$ complex all exhibit significant myocardial uptake following intravenous administration to animals, with the latter two rapidly providing excellent heart-to-blood ratios (*1,5–7*). Unfortunately, none of these compounds provide the myocardial retention of ^{68}Ga radioactivity that is needed to allow exploitation of the ^{68}Ga half-life through "slow" acquisition of high count images.

We report here a ^{68}Ga radiopharmaceutical, ^{68}Ga-[*bis*(4,6-dimethoxysalicylaldimino)-*N,N'-bis*(3-aminopropyl)ethylenediamine]$^+$ ($^{68}Ga[(4,6\text{-}MeO_2sal)_2BAPEN]^+$; see Fig. 1), that exhibits significant myocardial uptake upon intravenous injection followed by prolonged myocardial retention of the gallium radiolabel.

MATERIALS AND METHODS

The ligand precursors, 4,6-dimethoxysalicylaldehyde and *N,N'-bis*(3-aminopropyl)ethylenediamine, were purchased from Aldrich Chemical Co. (St. Louis, MO). $Ga(acac)_3$ was purchased from Strem Chemical Co. (Newburgport, MA). Gallium-67-chloride in HCl solution was obtained from Nordion International, Inc., Kanata, Ontario and Mallinckrodt Medical, Inc., St. Louis, MO. Gallium-68-Cl_3 was obtained in 1 N HCl from an ionic $^{68}Ge/^{68}Ga$ SnO_2 generator (*9*) purchased from DuPont/New England Nuclear, N. Billerica, MA. Radiochromatograms were analyzed with a Berthold Tracemaster 20 Automatic TLC Linear-Analyzer. All animal studies were carried out in accordance with procedures approved by the appropriate institutional review committees.

Synthesis of $H_3[(4,6\text{-}MeO_2sal)_3BAPEN]$

The *tris*(salicylaldimine) was synthesized by the general condensation reaction of three molar equivalents of aldehyde with the tetraamine (*10*). To a solution of 1.00 g of 4,6-dimethoxysalicylaldehyde (5.49 mmol) in 15 ml of dry methanol, 0.32 g of *N,N'-*

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For correspondence or reprints contact: Mark A. Green, PhD, Division of Nuclear Pharmacy, Department of Medicinal Chemistry, 1333 Pharmacy Bldg., Purdue University, West Lafayette, IN 47907-1333.

bis(3-aminopropyl)ethylenediamine (1.84 mmol) in 15 ml of dry methanol was added. The mixture was refluxed for 20 min and then stirred until cooled to 25°C. The solvent was removed by rotary evaporation. The resulting yellow oil was dissolved in diethyl ether. Unreacted aldehyde immediately precipitated and was removed by filtration. The filtrate was cooled to 0°C for approximately 24 hr and the bright yellow product that precipitated was filtered and washed with cold diethyl ether (40% yield, melting point 70–71°C). ^{1}H-NMR at 500 MHz in deuterated chloroform with TMS as reference: δ(ppm) 1.81 (m, 8H) -CH_2-N-CH_2-; 2.53 (m, 4H)-C-CH_2-C-; 3.43 (m, 4H) = N-CH_2-; 3.70, 3.72, 3.76 3.78 (s, 18H) -OCH_3; 4.32 (s, 1H) -N-CH-N-; 5.73 (m, 4H) C_6H_2; 5.97 (m, 2H) C_6H_2; 8.28 (m, 2H); CH = N. IR (KBr disk) ν(C = N) 1625 cm^{-1}. The fast-atom bombardment mass spectrum in positive-ion mode (DTT/DTE matrix) showed $[M + H]^+$ at m/z = 667 for M = $C_{35}H_{46}N_4O_9$.

Synthesis of Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+I^-$

A solution of 110 mg of $Ga(acac)_3$ (0.3 mmol) in 10 ml of warm ethanol was added to 200 mg of H_3[(4,6-MeO_2sal)$_3$BAPEN] (0.3 mmol) in 10 ml of warm ethanol. The mixture was heated to reflux for 30 min and 50 mg of KI in 1 ml of water was then added to the hot ethanol solution. The solution was slowly cooled to room temperature. The product precipitated out of the solution as a white microcrystalline which became solid upon cooling (86% yield, decomposes without melting at 298°C). ^{1}H-NMR at 500 MHz in dimethylsulfoxide-d_6 with TMS as reference: δ(ppm) 1.90 (m, 2H), 2.55 (m, 2H), 2.62 (m, 2H), 2.95 (m, 4H), 3.32 (m, 4H), 3.63 (m, 2H) -CH_2-; 3.74, 3.77 (s, 12H) -OCH_3; 4.90 (br, 2H) -NH-; 5.85 (s, 2H), 5.93 (s, 2H) C_6H_2; 8.20 (s, 2H) -CH = N. IR (KBr disk) ν(C = N) 1605 cm^{-1}. The fast-atom bombardment mass spectrum in positive-ion mode (DTT/DTE matrix) showed $[M]^+$ at m/z = 569 for $[C_{26}H_{36}N_4O_6Ga]^+$.

Synthesis and Characterization of Radiolabeled Complexes

Gallium-68 was eluted from the generator with 1 N HCl and the HCl was evaporated by heating under a stream of N_2 in a borosilicate test tube. The residue was redissolved in ethanol containing 0.002% by weight acetylacetone. The no-carrier-added [^{68}Ga]-gallium(III) *tris*(acetylacetonate) solution was then transferred to a clean test tube and 0.5 mg of the *tris*(salicylaldimine) ligand (5 mg/ml EtOH) was added. The ethanol solution was mixed and then heated for 10 min in a 65°C water bath to ensure completion of the ligand exchange reaction. The reaction solution was then diluted to 5% ethanol with saline and filtered through a 0.2-μm sterile polytetrafluoroethylene filter to deliver a product suitable for intravenous injection. The ^{67}Ga-labeled compound was prepared by a similar procedure.

The radiochemical purity of the $^{67/68}$Ga[(4,6-MeO_2sal)$_2$-BAPEN]$^+$ was always found to exceed 99% using thin-layer chromatography on C18 silica gel plates eluted with methanol (R_f = 0.1) and by paper chromatography on Whatman #1 chromatography paper eluted with ethanol (R_f = 1.0). Uncomplexed Ga^{3+} and unreacted $Ga(acac)_3$ were found to remain at the origin (R_f = 0.0) with both of these chromatography systems. The cationic nature of the radiolabeled complex was demonstrated by cellulose acetate electrophoresis in citrate-phosphate buffer at physiological pH (6.5 ml of 0.1 *M* citric acid and 43.6 ml of 0.2 *M* Na_2HPO_4 diluted to 100 ml) (*11*).

Technetium-99m-labeled Cardiolite® was prepared according to the instructions included with the commercial hexakis(2-methoxy isobutyl isonitrile)technetium(I) chloride radiopharmaceutical kit. The radiochemical purity of the product exceeded 99%, as determined by following the quality control protocol described in the package insert (aluminum oxide chromatography plate eluted with ethanol).

The octanol/water partition coefficients, P, for the gallium and technetium radiotracers were measured by vortex mixing of 1 ml of 1-octanol and 1 ml of isotonic Tris buffer (pH 7.4) (*12*) with approximately 0.1 μCi of the radiolabeled gallium complex. Following centrifugation at >1200x g for 5 min, the octanol and aqueous phases were sampled and counted in an automatic well counter. The octanol phase from this partitioning was repartitioned (2x) with fresh buffer to ensure that trace hydrophilic ^{67}Ga or ^{99m}Tc impurities did not alter the calculated P values.

Rat Biodistribution Studies

Under ether anesthesia, 1 to 3 μCi (0.1–0.2 ml) of no-carrier-added ^{67}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ (or ^{99m}Tc-Cardiolite®) was administered by bolus injection with a 27-gauge needle into the femoral vein of ether anesthetized male Sprague-Dawley rats. The dose administered to each animal was quantitated by weighing the injection syringe on an analytical balance before and after injection. The ether anesthetized rats were killed by decapitation at the specified time intervals postinjection and the organs of interest were excised, blotted to remove surface blood and weighed. The tissue radioactivity was measured in an automatic gamma counter. A standard made up from a measured aliquot of a known mass of the injectate was counted along with the tissue samples for quantitation of the injected dose for each animal. Radiopharmaceutical biodistribution was then calculated as a percentage of the injected dose per gram of tissue and percentage of injected dose per organ for each sample. Blood was assumed to account for 7% of total body mass.

PET Imaging Study of a Normal Dog

A PET imaging study of a normal mongrel dog injected with ^{68}Ga[4,6-MeO_2sal)$_2$BAPEN]$^+$ was performed at Washington University School of Medicine. The dog was anesthetized with thiopental and chloralose intravenously, intubated and ventilated with normal air and positioned in the PET VI camera (*13*). A transmission scan for attenuation correction was obtained followed by consecutive ^{15}O-carbon monoxide and ^{15}O-water scans for determination of a myocardial perfusion image (*14,15*). After decay of the ^{15}O radioactivity to background levels, 20 mCi of ^{68}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ was administered to the dog as a bolus intravenous injection. Dynamic PET data were then collected for ten consecutive 1-min frames followed by eight consecutive 10-min static images. Gallium-68 PET images of the chest were reconstructed from each of the ten 1-min frames and each of the eight 10-min frames.

RESULTS AND DISCUSSION

Synthesis and Characterization of Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+I^-$

The *tris*(salicylaldimine) ligand precursor, H_3[(4,6-MeO_2sal)$_3$BAPEN], is a stable solid that is readily prepared as shown in Figure 1. Reaction of this *tris*(salicylaldimine) with $Ga(acac)_3$ in aqueous ethanol results in the formation of the cationic *bis*(salicylaldimine) complex, Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$, which was isolated and characterized as the I^- salt. In this complex, we expect the gallium(III) ion to be bound by the two imine and two

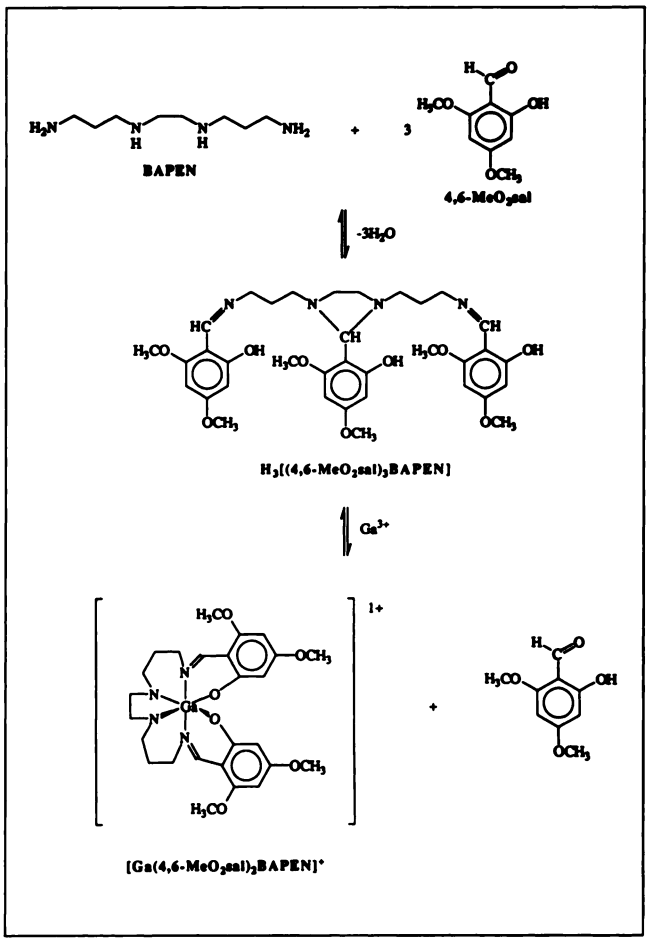

FIGURE 1. Synthesis and structural formula of the *tris*(salicylaldimine) ligand precursor and the cationic Ga(III) *bis*(salicylaldimine) complex. The Ga(III)[(4,6-MeO_2sal)$_2$BAPEN]$^+$ complex is drawn as the *cis* isomer, based on the reported structure of an Fe(III)*bis*(salicylaldimine) complex (*8*), although a *trans* arrangement of the phenolic oxygen donors may also be possible.

amine nitrogen lone pairs and by the two deprotonated phenolic oxygens, resulting in a hexadentate complex with an overall 1^+ charge. The ^{1}H-NMR spectrum of the gallium complex confirms loss of the bridging imino group, as does the FAB mass spectrum, which shows the expected parent ion peak due to Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$. Similar chemistry is known in the literature; the *tris*(salicylaldimine) of triethylenetetraamine reacts with metal ions in aqueous solution to give *bis*(salicylaldimino) complexes in which the bridging imino group has been lost by hydrolysis (*7,10*). The cationic Ga(III) complex of *bis*(salicylaldimino)triethylenetetraamine has previously been prepared by this method (*7*) as well as by the direct in situ reaction of $GaCl_3$ with triethylenetetraamine and two equivalents of salicylaldehyde (*16*).

The no-carrier-added ^{68}Ga and ^{67}Ga complexes of the [(4,6-MeO_2sal)$_2$BAPEN]$^{2-}$ ligand were similarly prepared by reaction of radiolabeled Ga(acac)$_3$ with the *tris*(salicylaldimine) in ethanol. The radiochemical purity of the ^{67}Ga and ^{68}Ga radiotracers was found to exceed 99% by thin-layer chromatography. The cationic nature of the ^{67}Ga radiotracer was confirmed by cellulose acetate electrophoresis studies which showed migration of the ^{67}Ga radioactivity towards the cathode. Although the complex is cationic, it is also quite lipophilic with a log P = 1.68 ± 0.04 (n = 3).

Rat Biodistribution Studies

The biodistribution of ^{67}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ in rats from 1 min to 2 hr following intravenous injection is shown in Table 1. The compound shows significant heart uptake and prolonged myocardial retention of the ^{67}Ga radiolabel. One percent of the injected dose is found in the heart at 1 min and remains there at 2 hr postinjection. The radiolabel clears rapidly from the blood to give excellent heart-to-blood ratios by 5 min postinjection (13.3 ± 2.2)

TABLE 1
Biodistribution of ^{67}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ in Rats*

Organ	Percentage of injected dose per organ†					
	1 min	5 min	15 min	30 min	60 min	120 min
Blood	8.49 ± 0.41	1.54 ± 0.18	0.96 ± 0.04	0.63 ± 0.02	0.43 ± 0.04	0.33 ± 0.02
Heart	1.04 ± 0.12	1.08 ± 0.15	0.87 ± 0.10	0.87 ± 0.10	1.02 ± 0.09	0.86 ± 0.11
Lungs	0.92 ± 0.21	0.63 ± 0.07	0.55 ± 0.06	0.45 ± 0.07	0.53 ± 0.02	0.52 ± 0.08
Liver	43.0 ± 3.6	39.5 ± 2.6	21.8 ± 2.7	11.7 ± 1.7	6.26 ± 0.95	3.10 ± 0.19
Spleen	0.38 ± 0.06	0.35 ± 0.10	0.28 ± 0.08	0.22 ± 0.08	0.26 ± 0.03	0.22 ± 0.06
Kidney (1)	5.26 ± 0.69	4.79 ± 0.25	3.63 ± 0.41	2.76 ± 0.03	2.72 ± 0.26	2.17 ± 0.17
Brain	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Heart-to-Blood‡	2.3 ± 0.4	13.3 ± 2.2	18.1 ± 0.9	29.6 ± 4.2	41.0 ± 4.4	45.6 ± 4.0
Heart-to-Lung‡	1.9 ± 0.2	2.6 ± 0.3	3.0 ± 0.5	2.8 ± 0.3	3.03 ± 0.12	3.3 ± 1.3
Heart-to-Liver‡	0.32 ± 0.03	0.34 ± 0.04	0.56 ± 0.10	1.05 ± 0.25	2.14 ± 0.12	4.1 ± 0.4

*Following bolus intravenous administration to male Sprague Dawley rats (178–217 g).
†Values at each time point represent the mean and standard deviation of data collected for four rats (seven rats at 1 and 5 min).
‡Ratios were calculated from the percentage of the injected dose per gram of tissue.

TABLE 2
Biodistribution of Cardiolite® in Rats*

Organ	Percentage of injected dose per organ†			
	1 min	5 min	30 min	60 min
Blood	2.73 ± 0.27	1.12 ± 0.25	0.29 ± 0.04	0.14 ± 0.01
Heart	1.44 ± 0.13	2.01 ± 0.61	1.86 ± 0.04	1.76 ± 0.07
Lungs	1.80 ± 0.37	1.58 ± 0.27	0.66 ± 0.06	0.42 ± 0.11
Liver	10.1 ± 2.0	14.2 ± 4.1	8.4 ± 2.0	4.8 ± 1.9
Spleen	0.61 ± 0.18	0.43 ± 0.24	0.36 ± 0.02	0.27 ± 0.04
Kidney (1)	5.7 ± 0.6	3.9 ± 1.1	1.54 ± 0.09	1.33 ± 0.15
Brain	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Heart-to-Blood‡	11.1 ± 0.2	36.6 ± 3.5	140.0 ± 42.0	208.0 ± 25.0
Heart-to-Lung‡	1.3 ± 0.2	1.8 ± 0.2	4.5 ± 0.4	6.5 ± 1.7
Heart-to-Liver‡	1.7 ± 0.1	2.1 ± 1.4	3.3 ± 0.9	5.3 ± 1.7

*Following bolus intravenous administration to male Sprague Dawley rats (233–275 g).
†Values at each time point represent the mean and standard deviation of data collected for three rats.
‡Ratios were calculated from the percentage of the injected dose per gram of tissue.

and approaches a heart-to-blood ratio of 50:1 at 2 hr postinjection (Table 1). As would be expected with a lipophilic tracer, a substantial fraction of the injected dose is taken up by the liver. Radioactivity is then slowly cleared into the bile. The resulting heart-to-liver and heart-to-lung ratios (Table 1) appear acceptable for PET imaging of the heart.

For comparison, the biodistribution of Cardiolite® (log P = 1.08 ± 0.04, n = 3) was similarly determined in rats (Table 2). The myocardial uptake of ^{67}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ is only slightly lower than the heart uptake found for the ^{99m}Tc myocardial perfusion agent, supporting the conclusion that ^{68}Ga complexes of this type may be useful in PET imaging of the heart. However, the myocardial uptake of ^{67}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ is still about three times lower than we have previously observed with ^{67}Ga[sal$_3$tame-O-iso-Bu] and related uncharged Ga-N_3O_3 Schiff-base radiotracers at 1 min postinjection (*5*). Thus, we believe it may be possible to prepare structural derivatives of Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ that exhibit substantially improved heart uptake while maintaining the desirable myocardial retention exhibited by this lead compound.

In comparing the distribution and pharmacokinetics of ^{67}Ga[(4,6-MeO_2-sal)$_2$BAPEN]$^+$ and Cardiolite® in tissues other than the heart, one sees that the gallium tracer clears from the blood somewhat more slowly than the technetium radiopharmaceutical (Tables 1 and 2). The lung uptake of the ^{67}Ga[(4,6-MeO_2-sal)$_2$BAPEN]$^+$ complex is slightly lower than the lung uptake seen with Cardiolite® in this animal model, whereas the liver uptake of the gallium tracer at 1 min postinjection is significantly higher than that observed for Cardiolite®. Thus, despite the fairly rapid clearance of ^{67}Ga radioactivity into the intestines, the heart-to-liver ratios for ^{67}Ga[(4,6-MeO_2-sal)$_2$BAPEN]$^+$ are not as good as those observed with the ^{99m}Tc radiotracer. The higher initial liver uptake of ^{67}Ga[(4,6-MeO_2-sal)$_2$BAPEN]$^+$ compared to Cardiolite® may result from the higher lipophilicity of the gallium complex.

Myocardial Imaging with ^{68}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$

The rat biodistribution data presented above suggest that the ^{68}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ complex could be useful as a PET radiopharmaceutical for imaging the heart. A PET imaging study was undertaken with a normal dog to further evaluate the potential of this tracer. Beyond 20 min postinjection, the heart was clearly delineated in the ^{68}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ images, with good heart-to-blood and heart-to-lung contrast (Fig. 2). Gallium-68 distribution in the myocardium was homogeneous, thus providing myocardial images similar to those observed in the ^{15}O-water perfusion study performed immediately prior to injection of the ^{68}Ga radiopharmaceutical. No clearance of

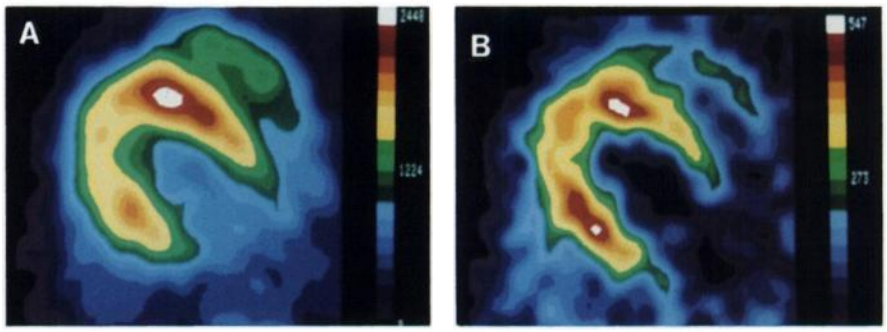

FIGURE 2. PET images of normal dog heart. (A) ^{68}Ga[(4,6-MeO_2sal)$_2$BAPEN]$^+$ image 40–50 min postinjection. (B) Blood-pool subtracted ^{15}O-water perfusion image obtained for reference immediately prior to administration of the ^{68}Ga radiopharmaceutical.

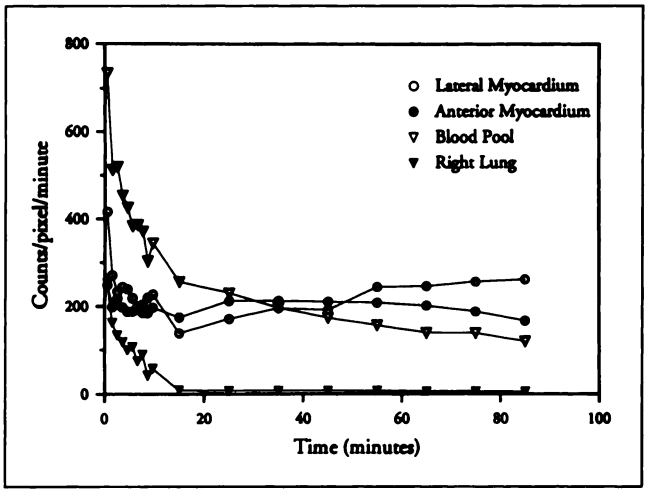

FIGURE 3. Tissue time-activity curves for $^{68}Ga[(4,6\text{-}Me_2O_2sal)_2BAPEN]^+$ following intravenous injection in a normal dog based on regions of interest from the PET study shown in Figure 2.

^{68}Ga radioactivity was observed from the myocardium over the 90-min imaging period (Fig. 3). This is consistent with the results from the rat biodistribution studies. However, blood-pool clearance in the dog was much slower than that observed in rats, with heart-to-blood ratios exceeding unity only beyond 20–30 min postinjection (Fig. 3). Nevertheless, these results indicate that cationic ^{68}Ga complexes of hexadentate $N_4O_2^{2-}$ Schiff-base ligands merit further investigation as agents for imaging the heart with PET.

CONCLUSION

Cationic ^{68}Ga(III) complexes with $N_4O_2^{2-}$ Schiff-base ligands appear promising as radiopharmaceuticals for PET imaging of the heart. The $Ga^{III}[(4,6\text{-}Me_2O_2sal)_2BAPEN]^+$ complex radiolabeled with ^{67}Ga and ^{68}Ga exhibits significant myocardial uptake in animal models following intravenous injection accompanied by myocardial retention of the gallium radiolabel. Further study will be required to screen this and related tracers in other animal models (e.g., guinea pig) that have been found by others to be good predictors of radiopharmaceutical behavior in man (*17–19*). In addition, studies remain in progress to determine the chemical fate of this tracer in the myocardium and to determine how myocardial $^{67/68}Ga$ uptake is related to the rate of regional myocardial perfusion.

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