Technetium-99m-Tetrofosmin as a New Radiopharmaceutical for Myocardial Perfusion Imaging

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A new cationic complex, $[^{99m}Tc(tetrofosmin)_2O_2]^+$, where tetrofosmin is the ether functionalized diphosphine ligand 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane, has been synthesized and evaluated for potential use in myocardial perfusion imaging. The structure of the complex has been determined by x-ray crystallography of the ^{99}Tc analog. In comparison with previously reported ^{99m}Tc complexes of alkyl-phosphines, the tetrofosmin species shows substantially increased clearance from nontarget tissue, especially blood and liver. A freeze-dried kit formulation has been developed. The kit provides a product of high radiochemical purity up to 8 hr after reconstitution at room temperature.

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In the past decade, substantial effort has been directed to the identification of ^{99m}Tc cations for myocardial perfusion imaging. To a significant extent, the body of research has been driven by interest in exploiting the superior imaging characteristics of ^{99m}Tc over ^{201}Tl (*1*).

Although the first documented case of a ^{99m}Tc cation (*2*) was based on a nitrogen macrocycle, the pioneering studies in the potential application of ^{99m}Tc cations to heart imaging were largely based on phosphine complexes (*3–7*) and, to a lesser extent, on arsines (*3,4*). These early attempts to obtain clinically useful myocardial imaging agents were frustrated by the inadequacy of animal models to predict behavior of lipophilic cations in man (*8,9*). For Tc(V) diphosphine cations, the question of interspecies variability had not arisen; no Tc(V) cation with promising heart uptake in animal models had been identified (*8*).

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Other research groups have explored the potential of alternative ^{99m}Tc ligand combinations. These have been largely based on Tc(I), and include isonitriles (*10*), arenes (*11,12*) and phosphites (*13*). All the Tc(I) complexes based on phosphorus donor ligands (*13*) exhibited similar behavior in man to the Tc(I) complex $[^{99m}Tc(DMPE)_3]^+$ {DMPE = 1,2 bis(dimethylphosphino)ethane}. They clear from the blood very slowly and show high liver uptake and slow washout from the heart. The isonitrile complex $[^{99m}Tc(TBIN)_6]^+$ (where TBIN represents t-butylisonitrile), however, was shown to be unique amongst the Tc(I) complexes in that it cleared rapidly out of the blood. Subsequent development of the isonitrile concept gave rise to a new cation, ^{99m}Tc-hexakis-methoxyisobutylisonitrile (MIBI) which is being applied for myocardial perfusion imaging (*14*).

We have explored the possibility that the limitations encountered with ^{99m}Tc complexes of simple alkyl or aryl diphosphines might be overcome by the introduction of hetero-atomic functions to modify nontarget uptakes (*15,16*). We report here the pre-clinical development of one of these complexes $[^{99m}Tc(tetrofosmin)_2O_2]^+$, where tetrofosmin = 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane. Note: this ligand has been referred to in earlier publications by the code 'P53'.

MATERIALS AND METHODS

Ligand

The structure of the ligand tetrofosmin is shown in Figure 1. Details of the synthesis and characterization of the ligand have already been reported (*17*).

Preparation of ^{99m}Tc Complexes

For the purpose of determining tissue distribution in small animals and for subsequent gamma camera imaging in pigs and early volunteer studies in man, the $[^{99m}Tc(tetrofosmin)_2O_2)]^+$

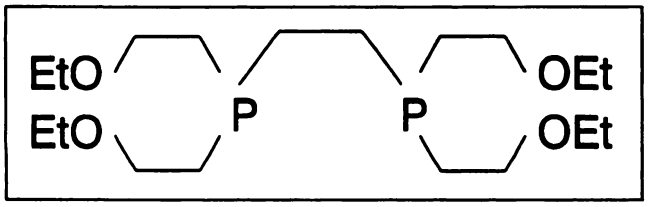

FIGURE 1. Structure of the phosphine ligand tetrofosmin: 1,2-bis[bis-(2-ethoxyethyl)phosphino]ethane.

complex was prepared as a liquid formulation. A freeze-dried formulation was subsequently developed.

All manipulations were carried out using standard procedures to ensure sterile, pyrogen-free preparations. Unless otherwise stated, oxygen was excluded from vials and solutions by nitrogen filling and purging, respectively.

Liquid Formulation $[^{99m}Tc(tetrofosmin)_2O_2]^+$. The tetrofosmin ligand (2 μl) was added to 10 ml saline containing 0.33 GBq $^{99m}TcO_4^-$ in a 10-ml vial sealed with a neoprene septum. The preparation was left to stand at ambient temperature for a period of not less than 2 hr and not more than 3 hr. The radiochemical purity was checked by HPLC or ITLC to ensure that $^{99m}TcO_4^-$ was less than 5% before filtration through a 0.2-μm Acrodisc (Gelman) filter into a septum-sealed vial. The filter was retained for microbiological testing. The filtered preparation was stable for up to 3 hr.

Lyophilized Formulation $[^{99m}Tc(tetrofosmin)_2O_2]^+$ (Myoview®, code PPN1011). The complex was prepared by the addition of 1 GBq ^{99m}Tc as sodium pertechnetate in 4–8 ml of saline to a lyophilized kit formulation in a 10-ml vial. Each vial contained 0.23 mg tetrofosmin, 30 μg stannous chloride dihydrate, 0.32 mg disodium sulphosalicylate and 1.0 mg sodium gluconate, pH adjusted before lyophilization and sealed under an atmosphere of nitrogen. The vial was shaken to dissolve the contents and allowed to stand at ambient temperature for 15 min before assay.

$[^{99m}Tc(DMPE)_3]^+$. Samples of $[^{99m}Tc(DMPE)_3]^+$ were prepared by literature methods (*7*).

Preparation of ^{99}Tc-Tetrofosmin

The long-lived ^{99}Tc-tetrofosmin analog was prepared by mixing $^{99}TcO_4^-$ (0.09 mmol) and tetrofosmin (0.45 mmol) in aqueous ethanol (6 ml). The mixture was purified by ion exchange chromatography (Sephadex SPC-25 eluted with 0.15 *M* $Li^+CF_3SO_3^-$) and a pink precipitate was isolated after mixing with saturated aqueous $NH_4[Cr(SCN)_4(NH_3)_2]$. The yield based on radioactivity was 65% and product was characterized by microanalysis (found %C 39.75, %H 7.46%, %N 6.98; calculated for $C_{40}H_{86}CrN_6O_{10}P_4S_4Tc$ %C 39.56, %H 7.14, %N 6.92). Diffraction quality crystals were obtained from cold ethanol.

Analytical Methods

High-Performance Liquid Chromatography (HPLC). HPLC was used to analyze the radiochemical purity of ^{99m}Tc and ^{99}Tc preparations. The HPLC system consisted of a Hamilton PRP-1 column (150 × 4.1 mm) eluted at a flow rate of 2.0 ml/min with a 17-min linear gradient from 100% 10 m*M* phosphate buffer (pH 7.5) to 100% tetrahydrofuran. The radioactivity detector (beta or gamma) was fitted to a rate meter and microcomputer programmed for peak integration.

Thin-Layer Chromatography. Measurement of the radiochemical purity of Myoview™ preparations was made using an ITLC/SG strip eluted with a 35:65 acetone:dichloromethane solvent mixture. Pertechnetate moves at the solvent front, and reduced hydrolyzed technetium and any hydrophilic complexes are retained at the origin. Radiochemical purity was measured as the percentage activity between R_f 0.3 and 0.8.

Gel Electrophoresis. Electrophoresis was used to determine the charge on cationic complexes. Studies were performed using a stationary phase of agarose A gel in 50 m*M* phosphate buffer, pH 7.0. Movement was determined relative to an anionic marker, bromophthalein blue (movement = - 10.0).

X-ray Crystallography. X-ray diffraction data were collected on a Philips PW 1100 diffractometer in the θ range 3–25° using Mo-K_α radiation from a weakly diffracting crystal measuring 0.48 × 0.12 × 0.10 mm^3. Crystal data are as follows: $C_{40}H_{86}CrN_6O_{10}P_4S_4Tc$, M = 1214.22, Triclinic, space group $P\bar{1}$(No. 2), a = 12.635(3) Å, b = 12.939(3) Å, c = 12.021(3) Å, α = 106.95(3)°, β = 113.11(3)°, γ = 60.83(3)°, U = 1566.38 Å^3, F(000) = 639, μ(Mo-K_α) = 0.64 mm^{-1}, Z = 1, D_c = 1.287 g cm^{-3}; final R 0.102 (R_w 0.0932) for 1409 data with $I/\sigma(I) > 2.5$

Biodistribution of ^{99m}Tc Complex in Rats

Wistar male and female rats (Interfauna UK, Huntingdon, Cambs) weighing between 150 and 200 g were injected with 0.1 ml of the test material via the lateral tail vein under light ether anesthesia. Animals were killed by exsanguination under ether anesthesia and the percentage of injected dose in the excreta and organs and tissues was determined by dissection. Samples of blood, muscle, bone, skin and fat were collected in pre-weighed containers. Other organs were removed intact, as listed in Tables 2, 3 and 4. All organs and tissues were assayed for radioactivity in a twin crystal automatic gamma counter. The accumulated activity in each organ or tissue was calculated as a percentage of the total dose.

For the liquid preparations, three male rats were killed at 2 min and 60 min postinjection. For the freeze-dried formulation, six rats were killed at 2 min, 60 min and 24 hr postinjection. The RCP of all preparations was checked prior to injection, and at all times the purity of the ^{99m}Tc complex was not less than 90%.

Biodistribution of ^{99m}Tc Complex in Guinea Pigs

Dunkin-Hartley albino male guinea pigs (Charles River UK, Margate, Kent) weighing between 230 and 265 g were injected with 0.1 ml of the test material via a front paw vein under local anesthesia (xylocaine 1%). Six guinea pigs were injected with each preparation, with three each being killed at 2 min and 60 min postinjection. Animals were killed by overdose of ether anesthesia followed by exsanguination.

The percentage of injected dose in the excreta and organs and tissues was determined by dissection and assay for radioactivity in an automatic gamma counter.

Gamma Camera Imaging in Minipigs

Gamma camera imaging studies were performed in male minipigs (Royal Veterinary College, Hatfield, Hertfordshire) weighing between 15 and 25 kg. Animals were sedated with azaperone (80 mg i.m. followed by 160 mg i.p.) and were then anesthetized using metomidate (75 mg i.v. initially).

One milliliter of the liquid $[^{99m}Tc(tetrofosmin)_2O_2]^+$ preparation containing approximately 5 mCi (185 MBq) ^{99m}Tc was administered via a Portex cannula into the jugular vein. The anesthetized minipig was placed supine on the face of a large field of view gamma camera and images were acquired using the anterior view at 2, 15, 30, 60, 90 min and 3, 5 and 24 hr postinjection.

Toxicology

Acute single-dose intravenous toxicity studies were performed in rats (six male and six female) using the liquid formulation of tetrofosmin. A dose equivalent to 400 times the human dose was administered, and animals were observed for 7 days following administration.

For the freeze-dried formulation of tetrofosmin (PPN1011), acute toxicity over a 14-day period was assessed in rats (five male and five female) and rabbits (three male and three female) at 0, 100 and 1,500 times the maximum single human dose. Repeat dose toxicity was assessed in rats (10 male and 10 female) and rabbits (5 male and 5 female) at 0, 10, 100 and 1,000 times the maximum human dose daily for 14 days.

Mutagenicity

The mutagenic potential of tetrofosmin was evaluated in three in vitro tests (Ames, mouse lymphoma and human lymphocyte) according to standard procedures and in one in vivo test (mouse micronucleus) modified to encompass two administrations of test article within 24 hr as a response to the rapid biological clearance of tetrofosmin.

RESULTS

Chemistry

The route of synthesis of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ using the liquid formulation was directly analogous to the method already established for the preparation of $[^{99m}Tc(DMPE)_2O_2]^+$ (*8*). Gel electrophoresis confirmed that the species formed was cationic; the ^{99m}Tc complex showed movement towards the cathode of +4.4 units relative to bromophthalein blue (−10 units) as a standard control. It was routinely possible to obtain preparations having radiochemical purity (RCP) greater than 90% by both HPLC and TLC with the liquid or freeze-dried formulations. A typical HPLC trace is shown in Figure 2. The $[^{99m}Tc(tetrofosmin)_2O_2]^+$ species has a retention time of 8.3 min.

Confirmation of the structure of the $[^{99}Tc(tetrofosmin)_2O_2]^+$ cation has been established by x-ray single crystal analysis of the ^{99}Tc analog in combination with established HPLC procedures to demonstrate equivalence of ^{99m}Tc and ^{99}Tc species.

A perspective view of the cation is shown in Figure 3. The structure shows the expected linear trans-oxo core, with the four phosphorus atoms of the two bidentate diphosphine ligands forming an exactly planar array. The main deviation from idealized octahedral geometry arises from the steric requirements of the five-membered ring formed by the diphosphine ligand resulting in P(1)-Tc-P(2) angles of 81.4(2)°.

The rate of formation of the $[^{99m}Tc(tetrofosmin)_2O_2]^+$ cation in the liquid formulation, which contains only tetrofosmin ligand and $^{99m}TcO_4^-$, is dependent upon the concentration of ligand (Table 1). At a concentration of 0.05 mg/ml, for example, reaction was only 50% complete after 6 hr standing at room temperature. The rate of reaction can be increased by increasing the concentration of ligand, by heating the preparation, or by a combination of both of these. At 120°C, and a tetrofosmin concentration of 0.05 mg/ml, the time taken to reach 50% conversion of $^{99m}TcO_4^-$ to $[^{99m}Tc(tetrofosmin)_2O_2]^+$ is reduced to less than 15 min. In comparison, the Myoview™ kit formulation enables the complex to be formed rapidly at room temperature with a ligand concentration as low as 0.03 mg/ml (Table 1). The kit preparation is stable for at least 8 hr after reconstitution.

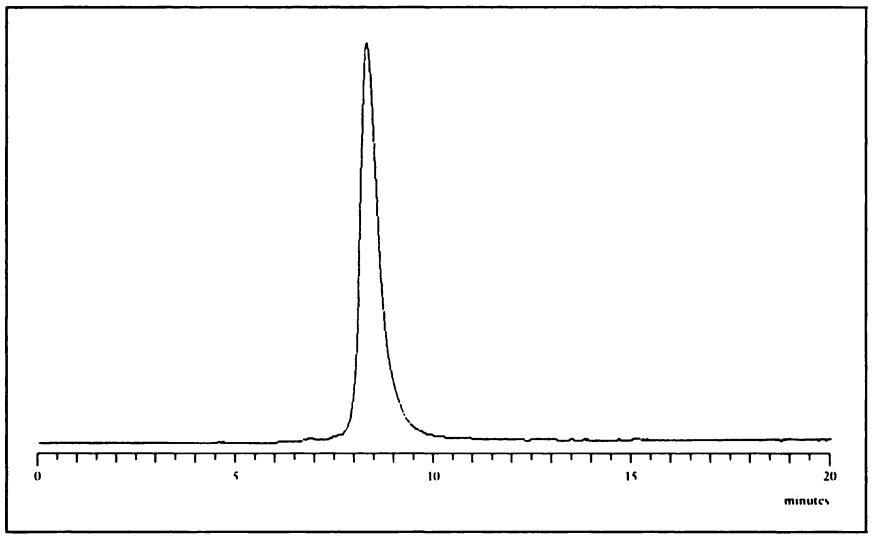

FIGURE 2. HPLC analysis of $[^{99m}Tc(tetrofosmin)_2O_2]^+$.

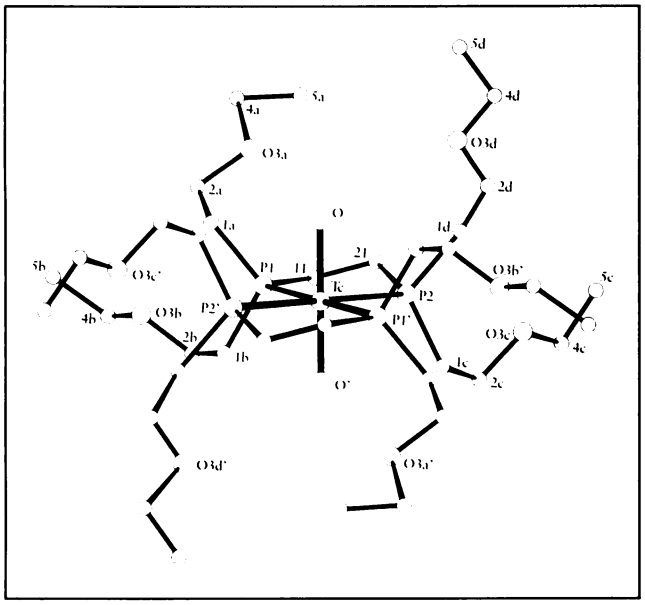

FIGURE 3. A perspective view of the $[Tc(tetrofosmin)_2O_2]^+$ cationic complex in the solid state showing the transoctahedral arrangement of the donor atoms. Selected bond lengths (Å): Tc-P(1) 2.476(8), Tc-P(2) 2.470(6), Tc-O 1.738(17).

Biodistribution and Safety Studies

The biodistribution of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ in rats and guinea pigs is shown in Tables 2 and 3, respectively. The complex shows good heart uptake with rapid clearance from blood and liver, and there is no significant uptake in lung tissue. Compared with the prototypical phosphine complex, $[^{99m}Tc(DMPE)_3]^+$, liver clearance, in particular, is greatly improved.

Figure 4 shows an anterior image taken 90 min postinjection in the pig. The complex is excreted primarily through the hepatobiliary system and, to a lesser extent, by the urinary route. Clinical studies in volunteers have since confirmed parallel behavior in man (*15*).

The detailed biodistribution in the rat of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ prepared from freeze-dried kits (Myoview™) is set out in Table 4 for timepoints up to 24 hr postinjection. The data are in good agreement with results obtained with earlier liquid formulations (Table 2).

TABLE 1
Dependence of Rate of Formation of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ on Concentration of Tetrofosmin Ligand

	Ligand concentration (mg/ml)	Time required for complete reaction (no detectable $^{99m}TcO_4^-$)
Liquid formulation	0.05	>6 hr
	0.1	2.5 hr
	0.2	2 hr
	1.0	>1 hr
	3.0	>0.5 hr
	6.0	0.5 hr
Kit formulation	0.03–0.06	<15 min

TABLE 2
Distribution of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ and $[^{99m}Tc(DMPE)_3]^+$ in Rats

Time post-injection	$[^{99m}Tc(tetrofosmin)_2O_2]^+$		$[^{99m}Tc(DMPE)_3]^+$	
	2 min	60 min	2 min	60 min
Heart	1.58 ± 0.09	1.53 ± 0.13	1.32 ± 0.01	1.18 ± 0.13
Blood	2.99 ± 0.34	0.54 ± 0.07	5.05 ± 0.38	0.58 ± 0.13
Muscle	30.8 ± 4.2	31.3 ± 7.9	27.4 ± 3.9	25.5 ± 9.1
Lung	1.51 ± 0.35	0.59 ± 0.19	2.34 ± 0.06	1.31 ± 0.09
Liver	13.5 ± 3.0	2.99 ± 0.51	21.8 ± 0.9	11.8 ± 0.8
Liver and GI	34.5 ± 1.5	42.2 ± 2.2	37.2 ± 0.7	44.6 ± 4.7
Kidney and urine	12.1 ± 1.5	13.3 ± 1.9	12.3 ± 0.6	15.1 ± 0.7

Mean ± s.d. of three animals, %ID in whole organ or tissue.

In acute toxicity studies in the rat, there was no evidence of toxicity of the "wet" formulation at a dose equivalent, on a body weight basis, to 400 times a single human dose.

The toxicology and mutagenicity of the freeze-dried formulation of tetrofosmin (PPN1011) has also been investigated. No mortalities occurred in any study. No toxicologically significant findings were made on single-dose administration of 1,500 times the maximum equivalent human dose, or on 14-day repeat dose studies at a level of 100 times the maximum human dose. Some changes indicative of liver effects (a slight reduction in organ weight) were noted on 14-day repeat dose studies at 1,000 times the maximum human dose in rabbits. Urinalysis and hematology were unremarkable in every case.

No significant mutagenic potential was seen in any of the test systems used.

DISCUSSION

In developing this complex, we sought to harness the myocardial affinity of ^{99m}Tc-diphosphine complexes and,

TABLE 3
Distribution of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ and $[^{99m}Tc(DMPE)_3]^+$ in Guinea Pigs

Time post-injection	$[^{99m}Tc(tetrofosmin)_2O_2]^+$		$[^{99m}Tc(DMPE)_3]^+$	
	2 min	60 min	2 min	60 min*
Heart	1.33 ± 0.14	0.99 ± 0.12	0.53 ± 0.08	0.38
Blood	3.11 ± 0.80	0.41 ± 0.07	28.6 ± 3.7	2.45
Muscle	30.0 ± 4.4	43.4 ± 28.4	11.0 ± 0.7	11.1
Lung	1.14 ± 0.19	0.47 ± 0.03	2.28 ± 0.42	0.66
Liver	11.6 ± 2.5	2.13 ± 0.56	38.0 ± 3.2	36.8
Liver and GI	38.7 ± 4.1	44.6 ± 2.8	48.0 ± 5.5	61.5
Kidney and urine	16.7 ± 1.2	16.1 ± 1.4	11.1 ± 0.3	15.7

* n = 2.
Mean ± s.d. of three animals, %ID in whole organ or tissue.

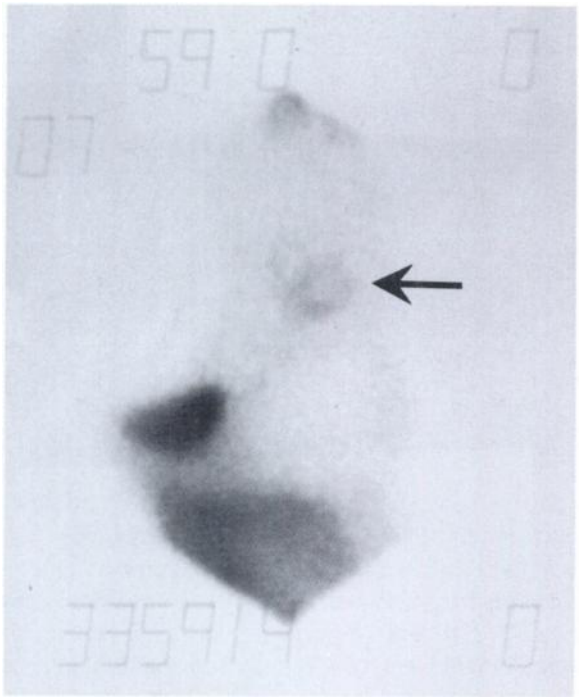

FIGURE 4. Anterior view in the pig imaged at 90 min after injection of $[^{99m}Tc(tetrofosmin)_2O_2]^+$. The position of the heart is indicated by an arrow.

at the same time, to eliminate the problems of poor background clearance. By the use of ether functional groups, we have managed to overcome the problems of retention in blood and slow hepatobiliary clearance which have generally been observed in studies in man of alkylphosphine complexes of technetium.

By use of a "wet" formulation, the ^{99m}Tc-dioxo complex of the ether functionalized phosphine, tetrofosmin, can be prepared in high radiochemical purity by reactions directly analogous to those previously used for preparation of $[Tc(diphosphine)_2O_2]^+$ complexes (*8*). The structure assigned to the tetrofosmin complex has been confirmed by x-ray crystal structure analysis and shows the expected trans-dioxo configuration.

The $[^{99m}Tc(tetrofosmin)_2O_2]^+$ species is the first example of a ^{99m}Tc-dioxo entity to have shown substantial myocardial uptake and retention, in combination with fast nontarget clearance, across a range of animal species, including man (*15*). In a review published in 1989, it was stated that no Tc(V) cation has been shown to accumulate in the myocardium of any species (*8*). The authors proposed that the polarity of the Tc = O linkages prevented myocardial uptake. This hypothesis may well explain the absence of heart uptake for Tc(V) cations based on simple alkyl or arylphosphines, but it is clear that it does not provide a basis for understanding the biological behavior of ether-functionalized phosphine complexes of technetium.

The freeze-dried formulation (Myoview®) addresses the inherent limitations of the reaction between $^{99m}TcO_4^-$ and diphosphines. At low ligand levels, the rate of reaction between $^{99m}TcO_4^-$ and diphosphines alone is relatively slow, as we have found using the simple wet formulations which contain only diphosphine ligand plus $^{99m}TcO_4^-$ in saline solution (see Table 1). The rate of reaction can be accelerated by increasing the ligand concentration or by heating. Because of the recognized thermodynamic lability of the Tc(V) dioxo diphosphine complexes (*8*), however, these approaches are likely to give rise to $[^{99m}Tc(tetrofosmin)_3]^+$. Using the Myoview™ formulation, the desired dioxo complex is formed rapidly in high radiochemical purity at ligand concentrations (at the highest volume of reconstitution) lower than 30 μg/ml. There is no formation of $[^{99m}Tc(tetrofosmin)_3]^+$ over the 8-hr life of the reconstituted kit.

TABLE 4
Biodistribution of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ Prepared from Myoview® Kits in Female and Male Wistar Rats

	Female			Male		
Time postinjection	2 min	60 min	24 hr	2 min	60 min	24 hr
Heart	1.75 ± 0.07	1.39 ± 0.09	0.11 ± 0.03	1.75 ± 0.09	1.49 ± 0.19	0.11 ± 0.02
Blood	1.68 ± 0.16	0.12 ± 0.03	0.02 ± 0.01	2.01 ± 0.17	0.22 ± 0.07	0.03 ± 0.01
Muscle	34.0 ± 11.0	26.3 ± 6.7	16.0 ± 2.0	24.0 ± 7.5	30.5 ± 11.0	17.1 ± 2.8
Lung	1.43 ± 0.19	0.37 ± 0.05	0.03 ± 0.01	1.68 ± 0.26	0.50 ± 0.20	0.04 ± 0.01
Liver	12.1 ± 3.1	1.66 ± 0.43	0.21 ± 0.02	15.0 ± 2.7	2.06 ± 0.58	0.26 ± 0.03
Small intestine	8.50 ± 2.17	3.67 ± 1.21	0.30 ± 0.12	7.41 ± 1.67	4.15 ± 1.04	0.35 ± 0.08
Small intestine contents	6.88 ± 1.81	38.0 ± 2.8	1.08 ± 0.51	8.16 ± 2.60	31.2 ± 6.6	1.10 ± 0.26
Large intestine	2.36 ± 0.65	1.47 ± 0.16	0.35 ± 0.11	2.82 ± 0.68	1.72 ± 0.19	0.36 ± 0.05
Large intestine contents	1.65 ± 0.33	4.10 ± 0.51	3.20 ± 1.06	1.81 ± 0.68	4.00 ± 0.61	2.52 ± 1.05
Feces	0.00 ± 0.00	0.01 ± 0.01	63.0 ± 3.3	0.00 ± 0.00	0.00 ± 0.00	60.3 ± 3.5
Kidneys	12.8 ± 1.4	2.57 ± 0.30	0.23 ± 0.02	12.8 ± 2.5	2.73 ± 0.71	0.25 ± 0.04
Total urine	0.13 ± 0.08	10.3 ± 0.8	13.2 ± 2.7	0.15 ± 0.06	9.52 ± 1.73	14.7 ± 2.0
Thyroid	0.19 ± 0.02	0.10 ± 0.02	0.03 ± 0.01	0.16 ± 0.07	0.12 ± 0.02	0.04 ± 0.01
Brain	0.05 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.05 ± 0.01	0.02 ± 0.01	0.00 ± 0.00
Fat	1.78 ± 0.62	1.02 ± 0.39	0.08 ± 0.02	1.99 ± 1.05	1.70 ± 0.97	0.53 ± 0.72

Mean ± s.d. of six animals; %ID in whole organ or tissue.

CONCLUSION

Based on the results of the experiments reported here, we have concluded that the structure of the technetium tetrofosmin complex is the trans-dioxo-bis(diphosphine)-technetium (V) cation $[Tc(tetrofosmin)_2)O_2]^+$. We have developed this complex of tetrofosmin for clinical use on the basis that it provides excellent myocardial uptake and retention with rapid background clearance. In addition, the lyophilized kit provides the convenience of room temperature reconstitution with 8-hr stability. Clinical trials are being conducted and will be reported in due course.

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