
Synthesis, Characterization, and Biodistribution of [$^{113\text{m}}\text{In}$]TE-BAT: A New Myocardial Imaging Agent

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In order to develop a new myocardial perfusion agent, new lipid-soluble complexes containing a net charge of +1 were evaluated. Synthesis, radiolabeling, characterization, and biodistribution of a unique indium complex, [$^{113\text{m}}\text{In}$]TE-BAT (tetraethyl-bis-aminoethanethiol), are described. The complex formation between In^{+3} and TE-BAT ligand is rapid, simple, and of high yield ($\geq 95\%$). This process is amenable to kit formulation. The complex has a net charge of +1 and an In/ligand ratio of 1:1. Biodistribution in mice shows higher heart uptake and longer retention as compared to ^{201}Tl . This complex, when labeled with ^{111}In , shows promise as a possible tracer for myocardial perfusion imaging.

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Several new technetium-99m ($^{99\text{m}}\text{Tc}$) agents for regional myocardial perfusion imaging have been reported (1-8). These agents are potential substitutes for thallium-201 (^{201}Tl) as the major radiopharmaceutical for routine nuclear medicine application. The $^{99\text{m}}\text{Tc}$ -labeled isonitriles, initially developed by Jones and Davison, have reached the final stage of clinical trial (1). The first agent of this series was [$^{99\text{m}}\text{Tc}$]TBI (t-butylisonitrile), which showed very high myocardial uptake and retention reflecting regional perfusion. However, the initial lung uptake and the subsequent high liver retention of [$^{99\text{m}}\text{Tc}$]TBI clearly indicates the need for further research and development (2). A new generation of isonitriles, [$^{99\text{m}}\text{Tc}$]MIBI (2-methoxyisobutyl nitrile) and CPI (2-carboxypropionylisonitrile) which show improved liver and lung washout, and the same high myocardial uptake and prolonged retention was reported (3-5). The [$^{99\text{m}}\text{Tc}$]MIBI is currently under phase II clinical trial. New boronic acid adducts of vicinyl dioximes, "cage" complexes of $^{99\text{m}}\text{Tc}$, (BATO, Squibb, SQ-30217) have been reported (6). Initial clinical study has also indicated that they may be useful as myocardial perfusion tracers (7). Another group of $\text{Tc} \cdot (\text{Arene})_2^+$ compounds, which is a "sandwich" complex, was reported (8). The clinical evaluation of these agents

in humans has not yet been reported; nevertheless, initial studies in animals showed very high myocardial uptake and prolonged retention.

Recent advances in chemistry of $^{99\text{m}}\text{Tc}$ complexes based on N_2S_2 ligands has dramatically enhanced our ability to predict the chemical structure of the final $^{99\text{m}}\text{Tc}$ complexes. This series of ligands forms strong complexes with $(\text{Tc} = \text{O})^{+3}$. The x-ray crystallography studies of several N_2S_2 complexes developed by ourselves and others (9-19) has confirmed the $(\text{Tc} = \text{O})^{+3}$ chemical state and the pyramidal core structure. Indium-111 (^{111}In) is a radionuclide with a $T_{1/2} = 2.8$ days and gamma rays of 172 and 247 keV, which are suitable for nuclear medicine imaging studies. Currently, the radionuclide [^{111}In]oxine is being used for white blood cell (20) and monoclonal antibody labeling. In order to investigate further the radiochemistry of indium, we have initiated a study using the N_2S_2 ligand, tetraethyl-bis-(aminoethanethiol) (TE-BAT), for complexing In^{+3} . This paper presents our data on synthesis, radiolabeling, characterization, and biodistribution of this unique In complex. For convenience in this study, $^{113\text{m}}\text{In}$ eluted from a tin-113m indium-113m ($^{113\text{m}}\text{Sn}$ - $^{113\text{m}}\text{In}$) generator was employed as the tracer. However, for imaging studies, ^{111}In is more suitable.

MATERIALS AND METHODS

General

The preparation of TE-BAT was achieved by a method reported previously (10). The only difference is that lithium

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aluminum hydride was employed for the last reduction step of diimine intermediate (19). The dimercapto hydrochloride salt of TE-BAT was precipitated and used for this study. Indium-113m was obtained by eluting a ^{113}Sn - $^{113\text{m}}\text{In}$ generator (Institute of Atomic Energy, Beijing, China) with 0.1 N HCl.

Radiolabeling

No-carrier-added [$^{113\text{m}}\text{In}$] chloride (1 mCi/ml) eluted with 0.1 N HCl was added to a test tube containing the BAT-TE ligand (2 mg) in 1 ml of water. In order to maintain the pH at 4–5, a simultaneous addition of a solution of 5% NaOH in the reaction mixture is needed. The mixture was vortexed and kept in a water bath at 80°C for 0.5 hr. The percent labeling yield was measured by thin layer chromatography (TLC) (Silica gel plate, developing solvent: acetone, $R_f = 0.6$). The radiochemical purity usually was over 96%. This material was used directly for animal studies. The effect of acidity and reaction time on the formation of this complex was determined by the same TLC technique.

Characterization of In-TE-BAT complex

Determination of composition. The composition of the complex, [$^{113\text{m}}\text{In}$]TE-BAT, was determined by a pH titration method (Radiometer, PH64). The formation of this complex follows the equation:



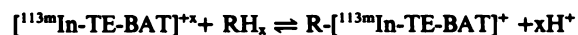
When [$^{113\text{m}}\text{In}$]TE-BAT is formed, two equivalents of [H^{+}] are released and can be titrated by a standardized sodium hydroxide solution (0.01N) (Fig. 1). The titration is performed under two different conditions (with or without [In^{3+}]): solution (A) containing 1.0 mg (0.391 mM) of TE-BAT in 7 ml of 1 mM HCl solution and solution (B) containing the same amount of ligand, 1.0 mg of TE-BAT in 3 ml of 1 mM HCl, and 4 ml of $\text{In}(\text{NO}_3)_3$ solution (1.9 mg in 50 ml of 1 mM HCl, 1.26×10^{-4} mM). Both of the solutions contain 0.1 N of NaCl (the same ionic strength). Based on the difference between titration curves A and B, the formation function can be calculated (21)

$$\bar{n} = \frac{[{}^{113\text{m}}\text{In-TE-BAT}]}{[\text{T}_M]},$$

where $[\text{T}_M]$ = total concentration of In^{3+} . At the endpoint of titration the formation function \bar{n} approaches unity, if the In/ligand ratio is equal to one.

Determination of net charge. Determination of net charge of this complex was achieved by the ion exchange method

(21). Ion exchange resin (cation, 10 mg/each experiment) was placed in a test tube together with a solution of [$^{113\text{m}}\text{In}$]TE-BAT (5 ml, at pH 0.9–2.3). The mixture was shaken for 1 hr. The resin and the solution were separated. The residual radioactivity in the solution was measured and the distribution coefficient (D) was calculated by counts in resin/counts in solution.



RH: Cation exchange resin

The equilibrium constant = K

$$K = \frac{\text{R}-[{}^{113\text{m}}\text{In-TE-BAT}]^{+}[\text{H}^{+}]^x}{[{}^{113\text{m}}\text{In-TE-BAT}]^{+x}[\text{RH}_x]}.$$

Distribution Coefficient = D

$$D = \frac{\text{R}-[{}^{113\text{m}}\text{In-TE-BAT}]^{+}}{[{}^{113\text{m}}\text{In-TE-BAT}]^{+x}}$$

$$\log D = \log K + \log[\text{RH}_x] + x\text{pH} \quad \log D = x\text{pH} + C.$$

The relationship between log D and pH is a straight line and the slope, x, is equal to the net charge of the complex.

Biodistribution in Mice

Biodistribution of [$^{113\text{m}}\text{In}$]TE-BAT was studied in male mice (18–22 g) which were allowed access to food and water ad lib. Saline solution containing [$^{113\text{m}}\text{In}$]TE-BAT in a volume of 0.1 ml was injected directly into the tail vein. Mice were killed (at various time points, 2 min to 1 hr, postinjection) by cardiac excision under ether anesthesia. The organs of interest were removed and counted using a well gamma counter. Percent dose per organ was calculated by comparison of tissue counts to suitably diluted aliquots of injected material. Total activities of blood and muscle were calculated assuming that they are 7% and 40% of total body weight, respectively.

RESULTS

Characterization of [$^{113\text{m}}\text{In}$]TE-BAT

Effects of acidity. It is well known that $\text{In}(\text{OH})_3$ is the predominant form when the pH of the reaction is higher than 5. The formation of the complex was evaluated at various pHs to determine the optimum conditions for labeling. The results shown in Figure 2 suggest that the labeling yield reaches a plateau between pH 4 and 5. At higher pH, precipitation of the ligand,

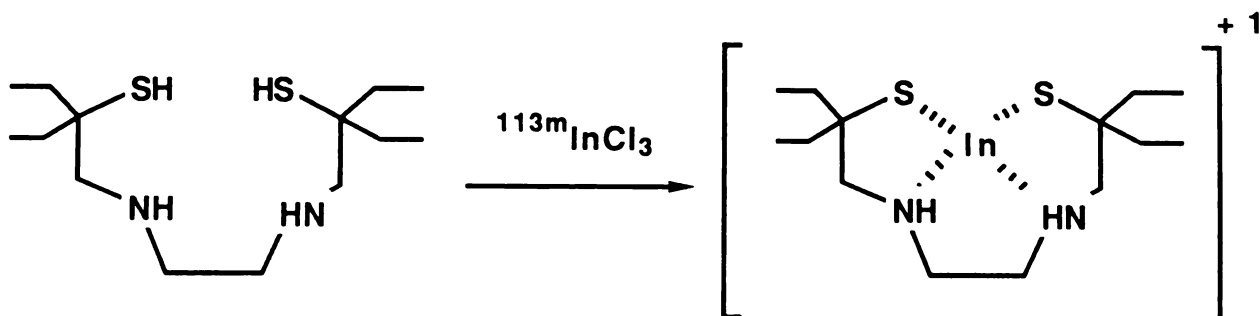


FIGURE 1
Chemical equation for the formation of [$^{113\text{m}}\text{In}$] TE-BAT.

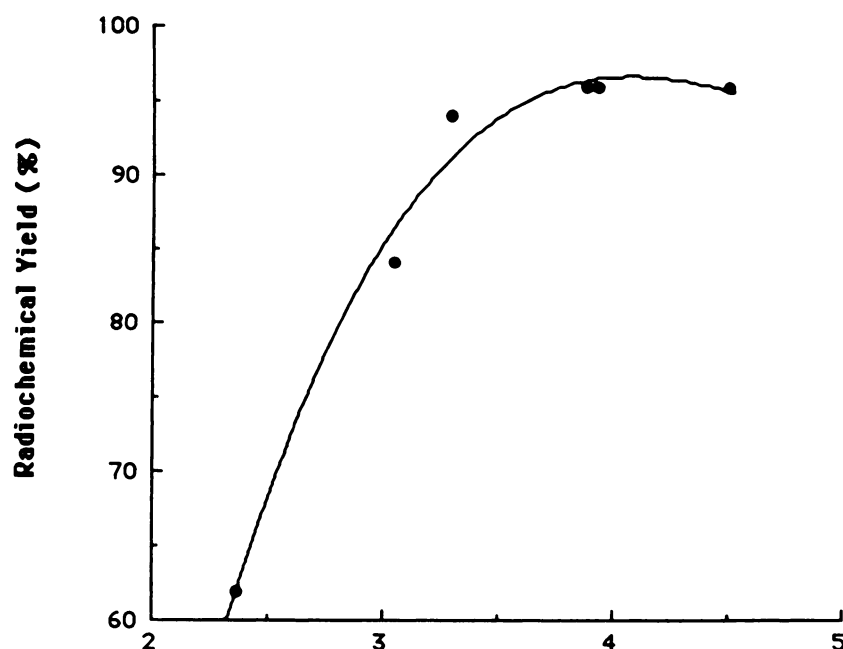


FIGURE 2
Effects of pH on the formation of $[^{113m}\text{In}]$ TE-BAT. The optimum pH range is between 4 and 5.

owing to its limited solubility in water, is observed (Fig. 2).

Reaction time. At pH 4, the labeling yield was evaluated at various reaction times. The formation of the complex reaches a plateau at 20 min (Fig. 3). Prolonged heating appears to have no significant effect on the labeling yield.

Determination of composition of In-TE-BAT. As indicated in Figure 1, the formation of no-carrier-added ^{113m}In -TE-BAT produces two hydrogen ions. After the complexation, the pH of the reaction solution decreases. The decrease of pH is stoichiometrically proportional

to the formation of the complex. This change can be measured by using acid-base titration techniques. The titration curves for the TE-BAT ligand at the same concentration with (B) and without (A) the presence of indium metal ion are represented in Figure 4. From this figure the concentration of $[\text{H}^+]$ can be calculated. The ionic strength of the solutions, under which curves A and B are generated, is the same, except that solution B contained In^{+3} (1.00×10^{-4}). At the same pH value, curves A and B show that a different volume of sodium hydroxide is consumed. The difference is a reflection of complex formation, and can be employed to calculate

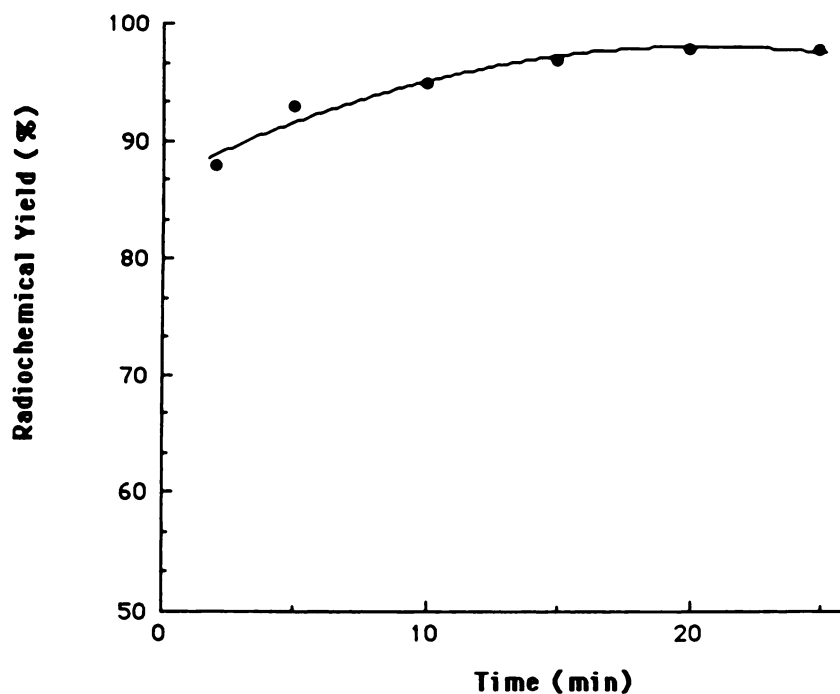


FIGURE 3
Effects of time on the formation of $[^{113m}\text{In}]$ TE-BAT. The formation of the complex reaches a plateau at 20 min.

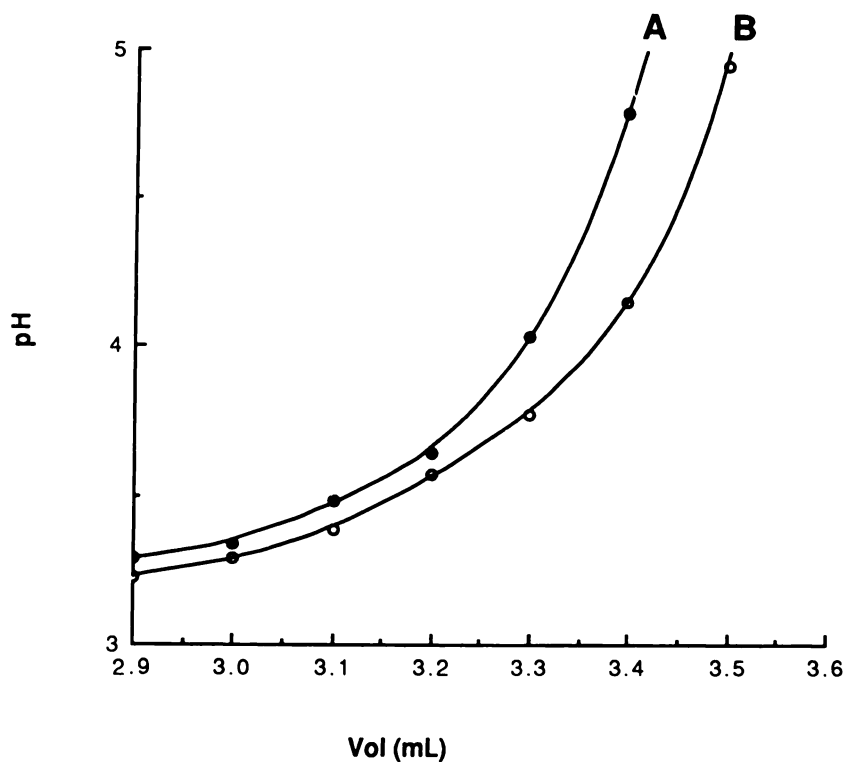


FIGURE 4
The titration curves of the ligand: TE-BAT, with (B) and without (A) the presence of indium metal ion ($1.00 \times 10^{-4} M$). The difference between these two curves at the same pH value is stoichiometrically proportional to the complex formation.

the concentration of the complex. Based on the titration curves and the stoichiometric relationship of hydrogen ion release and complex formation, the formation function (n) can be calculated. The relationship of formation function and pH is represented in Figure 5. This figure clearly indicates that the composition of the complex is 1:1, confirming the structure shown in Figure 1.

Determination of the net charge of the complex.

Using the ion exchange method, to determine the distribution constant (D) between resin and aqueous solution at various pHs, the net charge of the complex can be determined based on the following equation:

$$\log D = x\text{pH}_{\text{eq}} + C.$$

From Figure 6 the net charge, x , is determined to be 1.17. It is most likely that the net charge of this complex

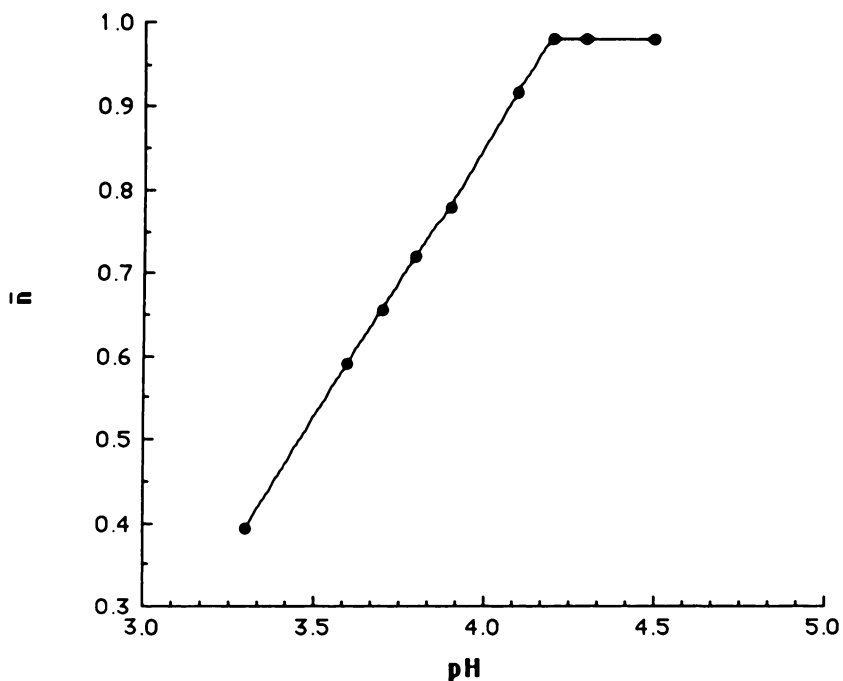


FIGURE 5
The relationship of the formation function (n) and pH. This figure indicates that the composition of the complex is 1:1, confirming the chemical structure shown in Figure 1.

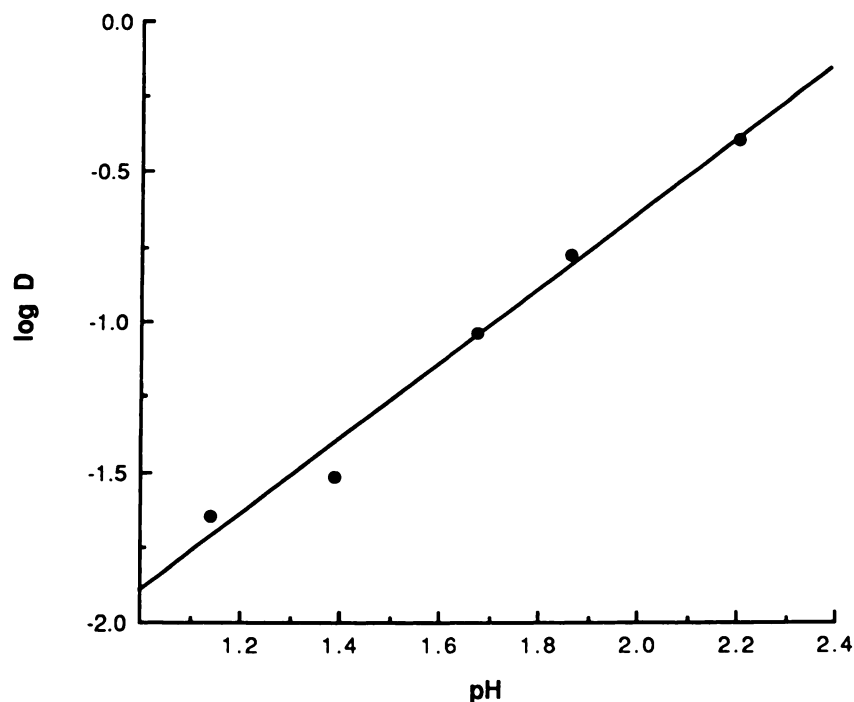


FIGURE 6
The relationship of the distribution constant (D) between resin and aqueous solution at various pHs. The net charge of the complex can be determined based on the slope of this straight line.

is +1. This is one more piece of evidence suggesting that the chemical structure in Figure 1 is correct.

Biodistribution in Mice

After an i.v. injection of [^{113m}In]TE-BAT in mice, a significant heart uptake (32.9% dose/g) at 2 min (i.v.) was observed. The heart uptake dropped to 22.5% dose/g at 15 min and 10.1% dose/g at 1 hr (Table 1 and Fig.

7). The uptake at these time points is higher than those reported for thallium-201 (^{201}Tl) and technetium-99m (^{99m}Tc) TBI (1, 21). The heart to lung, and heart to blood ratios for this complex are comparable or superior to those reported for ^{201}Tl and [^{99m}Tc]TBI. There is significant uptake in liver and lung which washes out with time. The ratios of heart/blood, heart/lung and heart/liver are reported in Table 1.

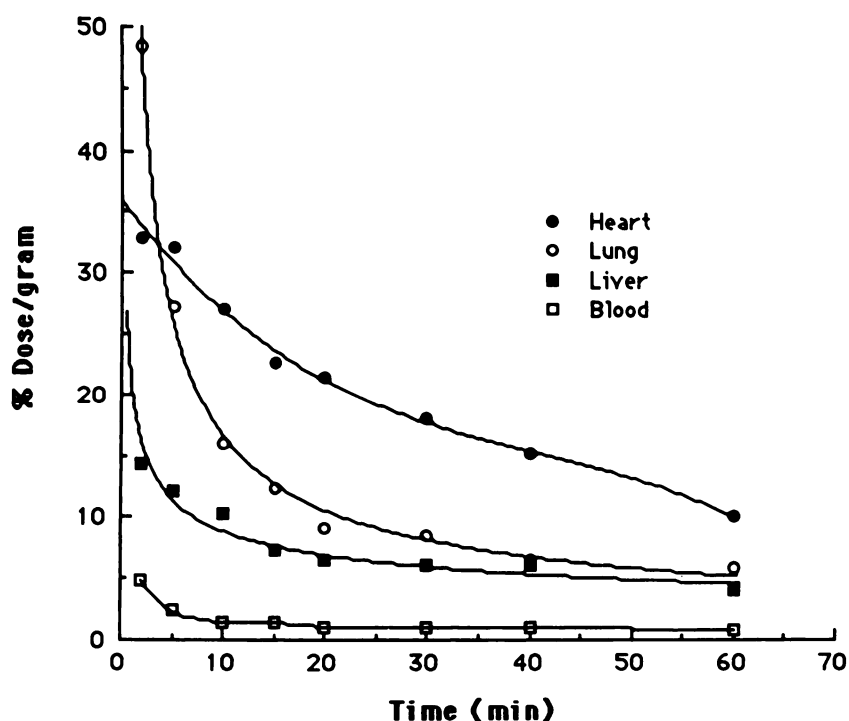


FIGURE 7
Biodistribution of [^{113m}In] TE-BAT in mice at different time points after i.v. injection.

TABLE 1
Biodistribution of [^{113m}In]TE-BAT*

	Time (postinjection)					
	2 min	5 min	10 min	20 min	30 min	60 min
Organ						
Heart	32.93 ± 8.07	32.14 ± 5.12	26.94 ± 3.80	21.34 ± 3.90	18.04 ± 2.48	10.13 ± 2.26
Blood	4.82 ± 1.53	2.33 ± 0.27	1.44 ± 0.22	1.08 ± 0.14	1.02 ± 0.17	0.81 ± 0.23
Lung	48.38 ± 9.64	27.21 ± 6.05	16.02 ± 3.23	9.17 ± 2.68	8.48 ± 2.98	5.82 ± 1.20
Liver	14.41 ± 2.03	12.13 ± 2.21	10.32 ± 2.81	6.52 ± 1.11	6.03 ± 1.99	3.97 ± 0.26
Time	2 min	5 min	10 min	20 min	30 min	60 min
H/blood	6.19	13.85	17.50	21.27	15.73	12.13
H/lung	0.50	1.00	1.64	2.25	2.05	1.72
H/liver	2.19	2.80	2.62	2.52	2.91	2.43

* Mean percent dose per gram ± s.d. (six mice).

DISCUSSION

The complex formation between In and TE-BAT ligand is very rapid, simple and occurs in high yield (≥ 95%). The high labeling efficiency and excellent purity of this labeling reaction means that it requires no further purification before being used in animal studies. It is possible that this process is amenable for kit formulation.

The labeling reaction is pH sensitive; the optimum pH range is between 4–5. This pH can be easily maintained by the addition of buffer solution and is, therefore, easily adaptable for a simple one step reaction. The net charge of the no-carrier-added [^{113m}In]TE-BAT is determined to be +1. In view of the fact that almost all of the myocardial perfusion imaging agents reported are +1 charge molecules, it is not surprising that [^{113m}In]TE-BAT, with the same net charge, also displays good heart uptake and retention. In mice, this agent displays fast myocardial uptake and rapid blood and lung wash-out; at 20 min postinjection the heart/blood and heart/lung ratios reach 21 and 2.25, respectively. At 1 hr postinjection the heart uptake still remains high: 10.13% dose/g. The heart uptake is comparable to that reported for ²⁰¹Tl and [^{99m}Tc]TBI (23). The biologic behavior of [^{113m}In]TE-BAT clearly suggests that this agent is potentially useful for myocardial perfusion imaging. It is necessary to determine the chemical structure by preparing “cold” In-TE-BAT complexes. In addition, further studies in primates and humans are needed, especially the “redistribution” of this agent in myocardial tissue, to fully characterize the physiological properties.

Technetium-99m-labeled myocardial perfusion agents are currently being developed, which could potentially replace the ²⁰¹Tl, the agent being used in the clinics at present. However, despite the superior physical characteristics of the ^{99m}Tc isotope (gamma ray 140

keV, T_{1/2} = 6 hr), the biologic behavior (no redistribution) of ^{99m}Tc isonitriles is different from ²⁰¹Tl (with delayed redistribution). The lack of redistribution for the ^{99m}Tc agents is being perceived as being less useful than ²⁰¹Tl, because the viability of damaged myocardium could not be studied effectively (24). This is probably one of the most controversial issues for myocardial perfusion imaging. The In-complex reported in this paper may have a different uptake and retention pattern when labeled with ¹¹¹In (T_{1/2} = 2.8 days); it may offer an alternative agent for evaluation of “redistribution” at 24 hr or even 48 hr after the initial injection.

In conclusion, ^{113m}In (III) chelates directly with TE-BAT to give a +1 charged complex. Biodistribution in mice showed significant heart uptake, a long retention time, high heart to blood ratios and low liver uptake. This agent, when labeled with ¹¹¹In, shows promise as a possible radiotracer for myocardial perfusion imaging.

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