# A New Tc-99m-Labeled Myocardial Imaging Agent, Hexakis(t-Butylisonitrile)-Technetium(I) [Tc-99m TBI]: Initial Experience in the Human

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**The cationic complex Tc-99m hexakis(t-butylisonitrile)technetium(I) (TBI) has been shown to concentrate in the myocardial tissue of several animal species. In the present preliminary study, the biodistribution of this material was examined in four normal subjects and in two patients with coronary artery disease. In three normal humans injected at rest, planar, tomographic, and gated myocardial images of high technical quality were obtained between 1 and 4 hr after injection. In one subject studied both at rest and during maximal exercise, the lung and heart activities were similar, whereas the liver-to-heart activity ratio was 3:1 at 60 min at rest compared with 1.8:1 with maximal exercise. In two patients with coronary artery disease, transient ischemia appeared as a perfusion defect up to 4 hr after injection at maximal exercise, and the image appeared normal when Tc-99m TBI was administered at rest. The images of areas of infarction appeared abnormal after injection at rest and after injection during exercise. Technetium-99m TBI is a promising myocardial imaging agent that may permit high-quality planar, gated, and tomographic imaging of myocardial ischemia and infarction.**

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In recent years, a number of Tc-99m-labeled agents have been reported that, based on their distribution in animals, appeared promising for myocardial perfusion imaging (*1–5*). When these radiotracers were tested in the human, however, the results were disappointing because of poor myocardial uptake (*6,7*). Several of the hexakis(alkylisonitrile)technetium(I) cations recently developed in these laboratories show marked cardiac uptake in a number of animal species (*8–10*). One of these, Tc-99m hexakis(t-butylisonitrile)technetium(I) (TBI), is promptly extracted into the myocardium in animals after intravenous injection, with a relatively constant concentration being maintained for at least several hours. After intracoronary injection there is no measurable myocardial washout for up to 1 hr (*11,12*). On the basis of previous experience, however, human studies are necessary to demonstrate the clinical potential of Tc-99m-labeled myocardial imaging agents. In this preliminary report, we describe the behavior of Tc-99m TBI in four normal subjects and in two patients with coronary artery disease.

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## METHODS

**Preparation of Tc-99m hexakis(t-butylisonitrile)-technetium(I).** The agent Tc-99m TBI was prepared by ligand exchange from a sterile pyrogen-free solution of the zinc bromide adduct of t-butylisonitrile and a standard preparation of Tc-99m glucoheptonate. The adduct $ZnBr_2(t\text{-}C_4H_9NC)_2$ was prepared from zinc bromide

and t-butylisonitrile in ether solution. The purity of the white crystalline product was verified by proton NMR spectroscopy and elemental analysis. Sterile pyrogen-free kits were then made by dissolving 1.56 g of the adduct in 50 ml isotonic saline and dispensing the solution in 1-ml aliquots into vials precooled with liquid nitrogen. The samples were kept frozen until used.

In order to synthesize Tc-99m TBI, up to 100 mCi of $^{99m}TcO_4^-$ generator eluent in ≤0.5 ml isotonic saline were added to a standard glucoheptonate kit. The thawed solution of the zinc bromide adduct (0.8 ml) was then added with shaking to the Tc-99m glucoheptonate, and the vial then placed upright in a boiling-water bath (90–100°C) for 15 min. After being allowed to cool, the fluid contents of the reaction vial were withdrawn and discarded, leaving 30–60% of the required Tc-99m TBI adhering to the walls of the vessel. The vial was rinsed gently twice with 10 ml of sterile water for injection, and 0.75 ml ethanol added to take up the complex. Sterile saline (2.25 ml) was then added to form an injectate that was 25% ethanol by volume, containing TBI in a Tc-99m concentration of ≥10 mCi/ml. An aliquot of 0.6 ml was taken for quality control.

Before injection, each TBI sample was assayed to ensure the purity of the product. High-pressure liquid chromatography was performed using a μ-Bondapak Radial Pak $C_{18}$ column having (10-μm particle size)* with 0.05 *M* $(NH_4)_2SO_4$ aq. (Solvent A) and methanol (Solvent B). Initial conditions were A:B = 100:0. Upon injection of a TBI sample, a 10-min linear gradient to a ratio A:B = 10:90 was applied, followed by a 5-min hold at the final condition. The solvent mixture was returned to A:B = 100:0 over 1 min, then held for a further 4 min in order to reequilibrate the column. The analysis was performed at ambient temperature, with flow rate 3 ml/min. Under these conditions, the retention times of Tc-99m TBI, $^{99m}TcO_4^-$, and Tc-99m glucoheptonate were 13.0–13.3, 2.2, and 1.5 min, respectively.

Reversed-phase, thin-layer chromatography (TLC) was carried out using $MKC_{18}F$ plates† developed with 50% 0.05 *M* $(NH_4)_2SO_4$ aq./50% MeOH. The $R_f$ of Tc-99m TBI and $^{99m}TcO_2 \cdot xH_2O$ in this system was 0.0, and those of $^{99m}TcO_4^-$ and Tc-99m glucoheptonate 0.89 and 1.00, respectively. The normal-phase procedure used GHLF silica gel plates‡ developed with 20% MeOH/80% $CH_2Cl_2$ ($R_f$ for Tc-99m TBI = 0.9; $R_f$ for $^{99m}TcO_4^-$ and $TcO_2 \cdot xH_2O$ = 0.0).

**Imaging studies.** Three male subjects, 48, 49, and 54 yr old and without clinical evidence of cardiopulmonary disease, were studied at rest. An intravenous injection of Tc-99m TBI (5–10 mCi) was performed slowly (15–30 sec) with the subject supine. Imaging of the chest and upper abdomen in the anterior position was begun immediately after injection, using a large-field Anger camera and digital computer. Images were acquired every minute for 1 hr. Venous blood samples were drawn at 2, 5, 10, 20, and 60 min after injection. At 60 min a urine sample was also obtained. Both blood and urine samples were counted in a gamma well counter to determine blood clearance and urinary activity.

Beginning at 1 hr, cardiac images were obtained in the anterior, 30° LAO, and 70° LAO projections, with 1 million counts collected in each image (~5 min per image). Four hours after injection, cardiac imaging was repeated.

Two to 3 hr after injection, SPECT imaging was performed in a 180° rotation using a standard reconstruction format (*13*). After SPECT imaging, ECG-gated studies were acquired in the anterior, 30° LAO, and 70° LAO positions. In one subject, imaging of the chest and upper abdomen was repeated at 2 and 4 hr after injection. In addition, whole-body imaging was performed 5 hr after injection.

Two normal subjects (one male who had been previously studied at rest, and one female) were studied during graded exercise using treadmill ergometry. At peak exercise, 10 mCi of Tc-99m TBI were injected intravenously, followed by 60 sec of additional exercise. Imaging was performed as described for the resting studies.

Biodistribution (through time-activity curves) was assessed from the 0- to 60-min data by taking an average activity per pixel in each region of interest, normalized to the injected dose (cpm/mCi, per pixel).

Two CAD patients were selected randomly: both had had myocardial infarctions, with abnormal exercise-tolerance tests, angina pectoris, and fixed (both patients) and transient (one patient) defects on exercise Tl-201 scintigraphy. Planar Tl-201 scintigraphy was performed immediately following graded treadmill exercise, and was repeated 3 hr later.

In both patients imaging with Tc-99m TBI was performed no later than 2 mo after the initial Tl-201 study. In neither patient was there change in clinical course or increase in symptoms. Ten mCi of Tc-99m TBI were injected at maximal treadmill exercise, which was continued for an additional minute. Cardiac imaging was performed as described above for the normal subjects. Imaging was repeated 1 wk later, after injection of 10 mCi of Tc-99m TBI at rest.

## RESULTS

Images of excellent technical quality were obtained from all subjects and patients. In the three subjects injected at rest, initial uptake in the lung was high (Fig. 1). By 60 min, the ratio of the heart to lung activity was 1.5:1. In one subject studied for 4 hr after injection, there appeared to be at least two components to the lung clearance, a fast compartment with a half-time of 10 min, and a slower one with a half-time of 4 hr. Assuming a two-compartment model, the fast component com-

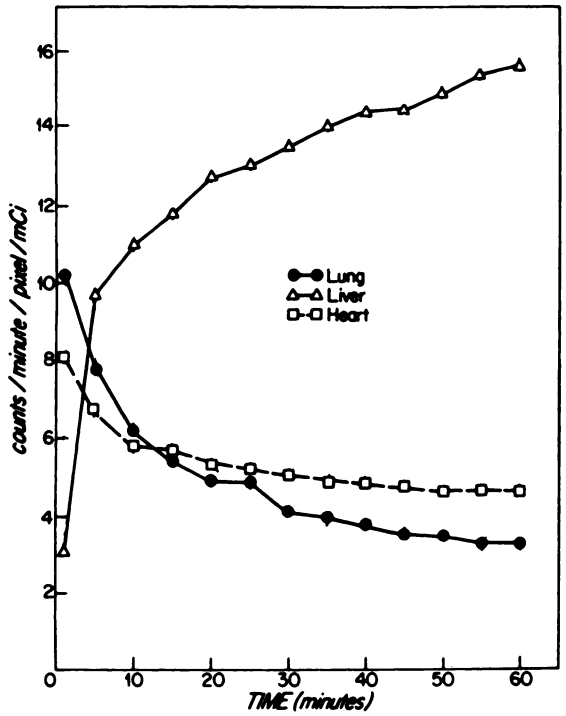


FIG. 1. Decay-corrected time-activity curves for lung, liver, and heart, after intravenous injection of Tc-99m TBI in three normal subjects at rest.

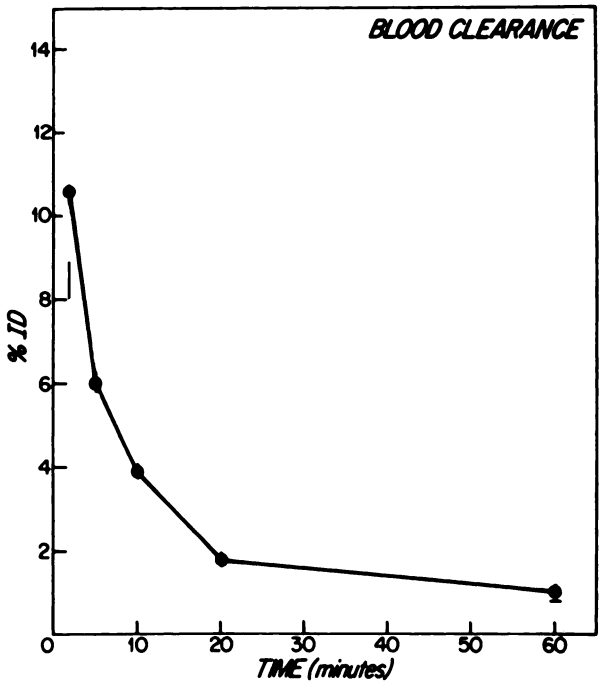


FIG. 3. Blood clearance of Tc-99m TBI in three normal subjects injected at rest.

prised 60% of the total initial lung uptake, and the slow component 40%.

Liver uptake rose steadily after injection (Fig. 1). By 60 min the liver-to-heart ratio was 3.4:1. Blood clearance was rapid, falling to less than 5% of the injected dose within the intravascular space by 10 min, and to 1% by 60 min (Fig. 2). The percentage of the radiotracer cleared through the urinary system at 60 min was extremely small in the three patients studied at rest (0.05%). In the subject injected at rest and during maximal exercise, heart and lung activities were the same for both studies (Fig. 3). On the other hand, liver activity was substantially less during maximal exercise.

Whole-body images obtained 5 hr after injection showed uptake primarily in the heart, liver, spleen, and skeletal muscles. Tracer appeared to be cleared primarily through the hepatobiliary system into the small bowel. Activity was also present in the kidneys, though little bladder activity was evident. Comparing the image ob-

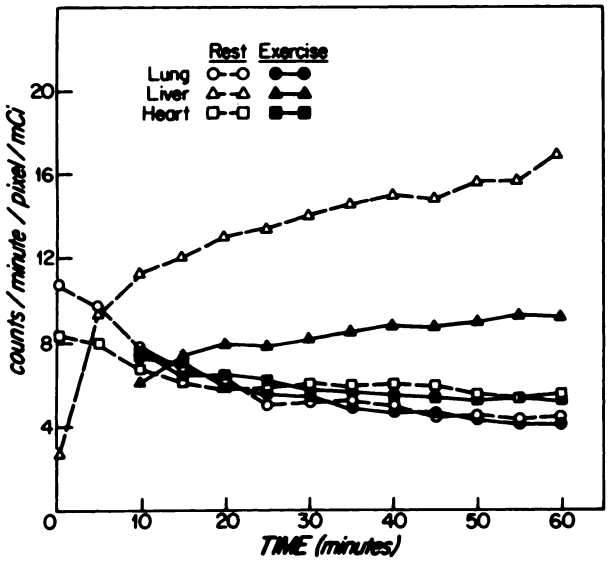


FIG. 2. Time-activity curves for lung, liver, and heart in one subject injected at rest and during maximal exercise.

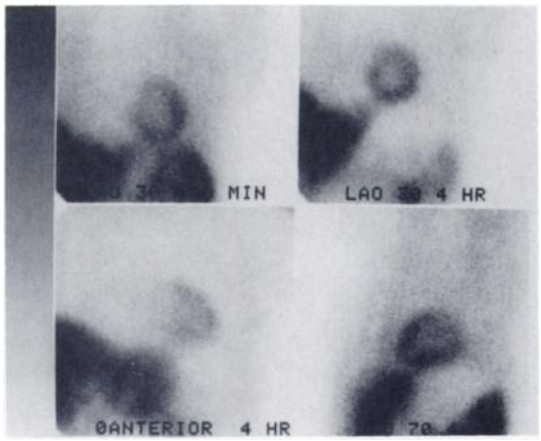


FIG. 4. Planar images obtained after injection of Tc-99m TBI at rest in normal subject: 30° LAO projection obtained 60 min after injection (upper left); 30° LAO projection at 4 hr after injection and after heavy meal (upper right); anterior projection at 4 hr after injection (lower right).

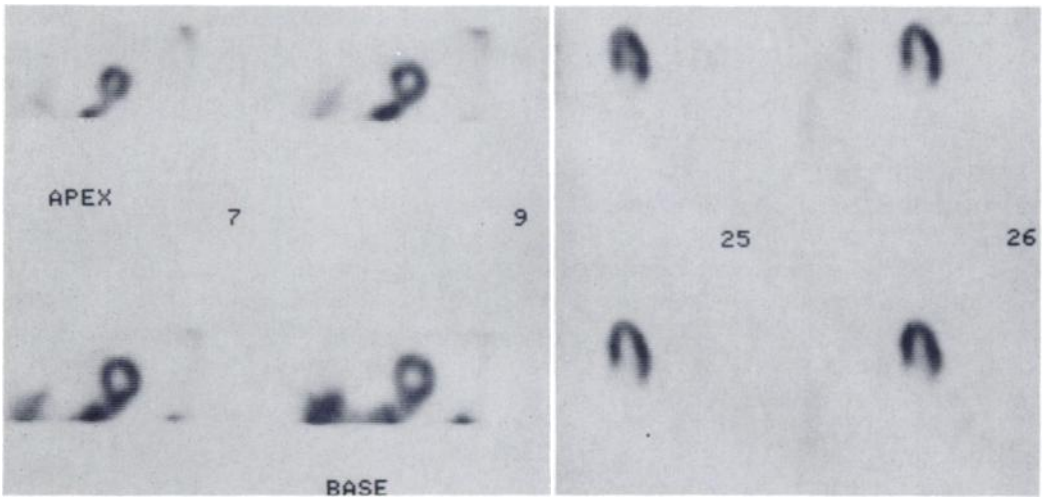


**FIG. 5.** Tomograms obtained in cucumber (short axis) projection (left) and horizontal long-axis projection (right) in normal subject. Uptake of Tc-99m TBI is uniform throughout left ventricle. Left lobe of liver is separated from inferoposterior wall of left ventricle on short-axis images. On horizontal long-axis images, apex of left ventricle is at top of horseshoe, septum to left, and lateral wall at right.

tained after maximal exercise with the baseline study, the former showed intense uptake within the leg muscles and substantially less liver uptake.

In the four subjects who had no evidence of cardiovascular disease, uniform uptake was seen throughout the left-ventricular wall. By 60 min, the concentration ratio between the left ventricle and the lungs was sufficiently high to permit image interpretation without the need for further background subtraction or contrast enhancement (Fig. 4). An increase in the heart-to-lung ratio at 4 hr improved image quality. The high concentration of activity in the liver did not affect imaging in the anterior and 30° LAO projections. Imaging in the 70° LAO projection was complicated in most cases by superimposition of the liver onto the inferoposterior wall of the left ventricle.

Tomographic images were of excellent quality in all subjects, permitting evaluation of all aspects of the left-ventricular wall, including the inferoposterior segment, with clear separation of the left lobe of the liver from the left ventricle (Fig. 5). Gated images demonstrated uniform contraction of the left and right ventricles in all normal subjects.

On Tl-201 myocardial scintigraphy, one of the patients with coronary artery disease had both a fixed defect involving the inferoposterior wall, due to previous myocardial infarction, and a transient defect involving the septum (Fig. 6). Technetium-99m TBI images obtained 1 and 4 hr after exercise demonstrated perfusion defects corresponding to both the fixed and reversible defects seen on the Tl-201 study. The increase in septal uptake on the Tc-99m TBI rest study corresponded to the redistribution into the septum observed with Tl-201. The second patient had an extensive prior myocardial infarction with a fixed defect visible on the Tl-201 study and corresponding defects seen with Tc-99m TBI after exercise. Tomographic imaging confirmed the presence of perfusion abnormalities in both cases. The abnor-

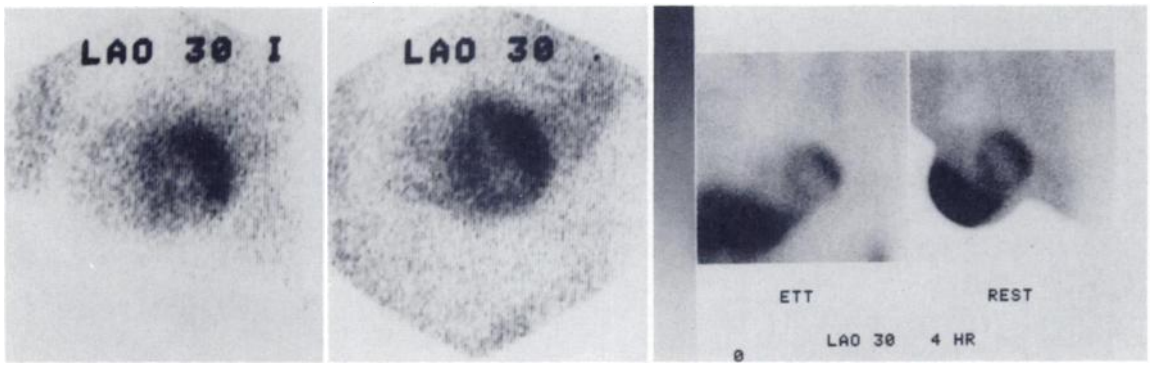


**FIG. 6.** Left: 30° LAO image, obtained immediately after exercise, shows markedly decreased uptake of Tl-201 in septum and apex. Center: Three hours later there is redistribution into septum and apex. Right: There is marked decrease in uptake in septum and apex on images obtained 4 hr after injection of Tc-99m TBI during maximal exercise (left). On images obtained 4 hr after injection of Tc-99m TBI at rest (right), there is some redistribution of tracer in heart.

mality appeared more extensive on tomography than with planar imaging.

## DISCUSSION

Myocardial scintigraphy with Tl-201 as thallous chloride has emerged as a routine test in the evaluation and management of patients with coronary artery disease (*14*). Thallium-201 has physical characteristics that make it less than optimal for myocardial imaging. For these reasons there is growing interest in the possibility of developing a Tc-99m-labeled agent to supplant Tl-201 so that the established advantages of technetium could be utilized in myocardial testing (*1–7*).

The biologic properties of a new class of technetium complex, the hexakis(alkylisonitrile)technetium(I) cations, have been reported by Jones et al., and indicate some potential as myocardial imaging agents (*8–10*). The most promising of these compounds showed uptake in the mouse heart ranging from 1.22 ± 0.12 to 1.24 ± 0.23% ID/organ from 5 min to 2 hr after intravenous administration. Successful cardiac imaging was also carried out with a number of other animal species, but previous experience with other technetium complexes emphasized that human trials would be necessary in order to determine whether the isonitrile complexes are clinically useful.

The present study demonstrates that planar and tomographic images of excellent technical quality can be obtained using Tc-99m TBI. In comparison with Tl-201, a substantially higher photon yield is possible, because of the higher injected dose permitted by the superior dosimetry of Tc-99m and because of weaker attenuation of the primary photons. As a result, the quality of the images obtained with Tc-99m TBI appears better than that obtained with Tl-201 despite the higher lung and liver uptakes found with Tc-99m TBI.

The biokinetics of Tc-99m TBI show high initial uptake and slow clearance from the lung, which precludes early imaging of the myocardium. In the studies reported here, satisfactory images were obtained at 60 min after injection. If Tc-99m TBI redistributes as rapidly as thallium, a substantial amount of redistribution will have occurred before imaging can begin. Animal studies suggest that the material may remain fixed in the myocardium for at least several hours after injection. There may, however, be myocardial uptake from delayed lung washout. Our preliminary results suggest that transient ischemia can, nevertheless, be detected as late as 4 hr after injection.

The relative amount of activity within the myocardium remains approximately the same whether the tracer is injected at rest or during maximal exercise. The failure of the heart activity to increase suggests that, as with thallium-201, there is a fall in the efficiency of tracer extraction at high flow rates.

Because the evidence indicates little or no alteration in the myocardial distribution of the tracer, it appears that two injections will be necessary to distinguish transient exercise-induced ischemia from irreversible myocardial damage. We are currently investigating whether the two injections can be given on the same day or whether the second study, to be performed at rest, must await substantial decay of the initial dose.

The agent Tc-99m TBI is only one of several alkylisonitrile complexes of technetium that Jones et al. have shown to exhibit myocardial uptake in animals (*8–10*). Furthermore, the substituent group in the isonitrile starting ligand can readily be altered, and not merely with simple alkyl or aryl groups. It is thus possible that among the many possible analogs of the class there may be other complexes with biological properties better suited to the intended use: for example, lower uptake and faster clearance from lung and liver, and perhaps also more rapid release from the myocardium itself to facilitate a dual-injection protocol.

Equally, the preparation of the Tc-99m TBI may need modification for routine use. The method described here produces an essentially pure sample of the technetium tracer in an injectable form that is virtually free of the starting materials. For this we relied on the physical property of the complex to adhere to the walls of the reaction vessel. The overall yield of injectable solution, however, is in the range 30–60%, which may be unacceptably low for a routine radiopharmaceutical preparation.

## FOOTNOTES

* Waters Associates, Milford, MA 01757.
† Whatman, Inc., Clifton, NJ 07104.
‡ Analtech, Inc., Newark, DE 19711.

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