Effect of Reperfusion and Hyperemia on the Myocardial Distribution of Technetium-99m t-Butylisonitrile

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Technetium-99m t-butylisonitrile ([^{99m}Tc]TBI) is a promising new radiotracer for myocardial imaging. Its myocardial uptake is sufficiently high in humans to permit planar, tomographic, and gated images of excellent technical quality. We studied the behavior of [^{99m}Tc]TBI in the dog at rest and under conditions of hyperemia and reperfusion in order to determine the relationship between [^{99m}Tc]TBI myocardial concentration and blood flow. After permanent occlusion of the left anterior descending artery, the correlation between the relative myocardial concentration of [^{99m}Tc]TBI and regional myocardial blood flow (RMBF) measured with radiolabeled microspheres was excellent. In a dog model of transient hyperemia, the concentration of [^{99m}Tc]TBI was directly related to blood flow but underestimated the degree of hyperemia. Technetium-99m TBI redistributed into transiently ischemic myocardium. The myocardial concentrations of [^{99m}Tc]TBI and thallium-201(^{201}Tl) in transiently ischemic myocardium were similar at 10 and 30 min following reperfusion and were significantly higher than blood flow prior to reperfusion. When [^{99m}Tc]TBI was injected into the left anterior descending artery, the washout was slow, falling to 78% of initial activity at 120 min after injection. In conclusion, [^{99m}Tc]TBI reflects regional myocardial blood flow accurately in ischemic and normal resting myocardium and underestimates blood flow at high flows. The rate of myocardial redistribution after reperfusion is similar for [^{99m}Tc]TBI and ^{201}Tl.

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A recently developed member of the hexakis (alkylisonitrile) technetium (I) family of cations, technetium-99m hexakis (t-butylisonitrile) technetium (I) ([^{99m}Tc]TBI), is taken up by the human myocardium in sufficient concentration to permit planar and tomographic images of excellent technical quality (*1,2*). Its myocardial distribution appears to reflect myocardial perfusion and is similar to the initial distribution of thallium-201 (^{201}Tl). Unfortunately, its initial uptake in the lung is high with slow clearance. As a result, myocardial images obtained earlier than 30–60 min after injection are of poor technical quality because of the high lung background. While delays of up to 1 hr would not affect the utility of [^{99m}Tc]TBI imaging in patients with suspected acute myocardial infarction (MI) or in situations where interventions were not needed, evaluation of transient ischemia would be limited if [^{99m}Tc]TBI behaved like ^{201}Tl with fairly rapid redistribution of the tracer after the completion of the exercise test. Initial results have suggested that [^{99m}Tc]TBI washes out very slowly from the myocardium and that defects due to transient ischemia may persist long enough to be detected on delayed images (*2,3*). If [^{99m}Tc]TBI is to replace or complement ^{201}Tl as a tracer of myocardial perfusion, its biodistribution pattern in regions of infarction and transient ischemia must be understood.

In this study, we examined the relationship between the distribution of [^{99m}Tc]TBI and myocardial blood flow in a canine model of ischemic and transiently hyperemic myocardium. We also studied the redistribution of this tracer following transient ischemia as well

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as the rate of myocardial washout after direct coronary injection.

MATERIALS AND METHODS

Preparation of Technetium-99m Hexakis (t-Butylisonitrile)-Technetium(I)

The agent [^{99m}Tc]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 [^{99m}Tc]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 nuclear magnetic resonance 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 1.0 ml aliquots into vials precooled with liquid nitrogen. The samples were kept frozen until used.

In order to synthesize [^{99m}Tc]TBI, up to 100 mCi of $^{99m}TcO_4^-$ generator eluate 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 added with shaking to the [^{99m}Tc]glucoheptonate, and the vial was 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 [^{99m}Tc]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 was 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 [^{99m}Tc]TBI in a ^{99m}Tc concentration of 10–20 mCi/ml. The specific activity of the [^{99m}Tc]TBI was 3.3×10^{-5} Ci/mmol.

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 Pac C18 column (10-μ particle size) with 0.05*M* $(NH_4)_2SO_4$ (Solvent A) and methanol (Solvent B). Initial conditions were A:B = 100:0. Upon injection of a [^{99m}Tc]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 re-equilibrate the column. The analysis was performed at ambient temperature, with a flow rate of 3 ml/min. Under these conditions, the retention times of [^{99m}Tc]TBI, $^{99m}TcO_4^-$, and [^{99m}Tc]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* $(NH_4)_2SO_4$/50% MeOH. The Rf of [^{99m}Tc]TBI and $^{99m}TcO_2 \cdot H_2O$ in this system was 0.0, and those of $^{99m}TcO_4^-$ and [^{99m}Tc]glucoheptonate were 0.89 and 1.00, respectively. The normal-phase procedure used GHLF silica gel plates developed with 20% MeOH/80% CH_2Cl_2 (Rf) for [^{99m}Tc]TBI = 0.9; Rf for $^{99m}TcO_4^-$ and $TcO_2 \cdot H_2O$ = 0.0).

Animal Preparation

Ten mongrel dogs of either sex weighing 18 to 21 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated, and ventilated with room air using a Harvard respirator. Cannulae were positioned in the left carotid artery for monitoring of the arterial blood pressure and for withdrawal of a reference blood sample for determination of regional myocardial blood flow (RMBF) and in the left jugular vein. A left thoracotomy was performed in the fifth intercostal space and a pericardial cradle was constructed. The left anterior descending coronary artery (LAD) was isolated proximal to the first major diagonal branch. A third catheter was placed in the left atrium for injection of radioactive microspheres for determination of regional myocardial blood flow.

Protocol A

In three of these dogs, the LAD was occluded using a Schwartz vascular clamp. In each case, this was associated with the development of a clearly visible region of epicardial cyanosis along the anterior surface of the left ventricle. Twelve minutes postocclusion, 3–5 mCi of [^{99m}Tc]TBI was injected intravenously, immediately followed by the intra-atrial injection of radioactive (scandium-46 or tin-113) microspheres (diam 8-10 μ), while an arterial reference blood sample was withdrawn at 15.3 ml/min using a Harvard withdrawal pump. Fifteen minutes postocclusion, monastral blue (1 ml/kg) was injected into the left atrium to facilitate identification of the ischemic myocardium. The blue dye circulates to the nonischemic myocardium; the ischemic coronary bed remains unstained. The anesthetized dogs were killed by intra-atrial injection of KCl (40-60 meq) and the hearts were excised. The atria and right ventricle were removed and the left ventricle was transversely sectioned from apex to base into 4–5 mm sections for analysis of myocardial blood flow and [^{99m}Tc]TBI activity. The slices were dissected radially from the following four zones of the heart using the monastral staining as a guide: (a) the center of the ischemic zone, (b) the peripheral zone of the ischemic area, (c) the normal zone adjacent to the ischemic area, and (d) the normal zone remote from the ischemic area. Each of these sections was further dissected into endocardial and epicardial portions of myocardium. This procedure yielded 32 samples, each weighing ~1g. The concentration of [^{99m}Tc]TBI was determined using a gamma well counter. The activity/g in the test sample was normalized by dividing it by the activity/g in the

remote normal myocardium. One week later, after the decay of ^{99m}Tc, the samples were recounted for calculation of regional myocardial blood flow, which was calculated using the formula: RMBF = $C_s \times (C_B/C_R)$ where RMBF = flow in the myocardial tissue sample (ml/min/g); C_S = counts in the myocardial tissue sample per gram of tissue; C_B = rate of withdrawal for the arterial reference blood sample, and C_R = total counts in arterial reference blood sample (*4*).

Protocol B

In three dogs, the relationship between regional myocardial blood flow and myocardial uptake of [^{99m}Tc]TBI was determined during hyperemia. The protocol was as described above except that the left anterior descending artery was reperfused 15 min postocclusion by abrupt removal of the Schwartz vascular clamp. Technetium-99m TBI was injected intravenously 5 min after reperfusion immediately followed by the atrial injection of radioactive microspheres. The dogs were killed 5 min after the injection of [^{99m}Tc]TBI with injection of KCl. Tissue dissection and measurement of radioactivity followed the protocol described above.

Protocol C

In two dogs, the rate of redistribution of [^{99m}Tc]TBI was determined and compared to that of ^{201}Tl. The LAD was occluded for 15 min with a Schwartz vascular clamp. Three to five millicuries [^{99m}Tc]TBI and 0.5 mCi of ^{201}Tl as thallous chloride were sequentially injected intravenously 12 min postocclusion, immediately followed by injection of radioactive microspheres into the left atrium. Reperfusion was instituted at 15 min postocclusion. The dogs were killed with injection of KCl 10 or 30 min after reperfusion. Tissue dissection and measurement of radioactivity followed the protocol described above except that ^{201}Tl activity/g was also determined by gamma well counting and the measurement of microsphere activity was delayed until after the radioactive decay of ^{201}Tl.

Protocol D

In two dogs, the washout of [^{99m}Tc]TBI from the myocardium was determined by injection 1–2 mCi of [^{99m}Tc]TBI directly into the left anterior descending artery. In this protocol, the LAD was not occluded. In vivo myocardial biopsies were obtained initially, at 20–30 min, at 50–60 min, and at 2 hr after injection. Sample sites were as close to the distal left anterior descending artery as possible, to minimize the effect of distance from the artery on the concentration of the tracer.

RESULTS

Protocol A

The correlation between regional myocardial blood flow and the relative concentration of [^{99m}Tc]TBI was excellent in zones of normal blood flow and in zones of ischemia [^{99m}Tc]TBI = 0.97 RMBF − 0.01; r = 0.98; s.e.e. = 0.07) (Fig. 1). The relationship between blood flow and [^{99m}Tc]TBI concentration was similar for subendocardial and subepicardial tissue (subendocardium [^{99m}Tc]TBI = 0.97 RMBF + 0.03; r = 0.98; s.e.e. = 0.07; subepicardium [^{99m}Tc]TBI = 1.0 RMBF + 0.01; r = 0.97; s.e.e. = 0.08).

Protocol B

In transiently hyperemic myocardium, the concentration of [^{99m}Tc]TBI was directly related to blood flow although, at high flows, the [^{99m}Tc]TBI concentration underestimated the degree of hyperemia (Fig. 2). Furthermore, the correlation between [^{99m}Tc]TBI concentration and myocardial blood flow was not as good in transiently hyperemic tissue as it was in ischemic and normal myocardium when the data from all three dogs were combined (r = 0.85; [^{99m}Tc]TBI = 0.49 RMBF + 0.18). When the data from each dog were analyzed separately, the correlation between [^{99m}Tc]TBI concentration and blood flow was excellent (Dog 1: r = 0.98, [^{99m}Tc]TBI = 0.26 RMBF + 0.56; s.e.e. = 0.09; Dog 2: r = 0.99; [^{99m}Tc]TBI = 0.56 RMBF + 0.31; s.e.e. = 0.21; Dog 3: r = 0.98; [^{99m}Tc]TBI = 0.28 RMBF + 0.63; s.e.e. = 0.08).

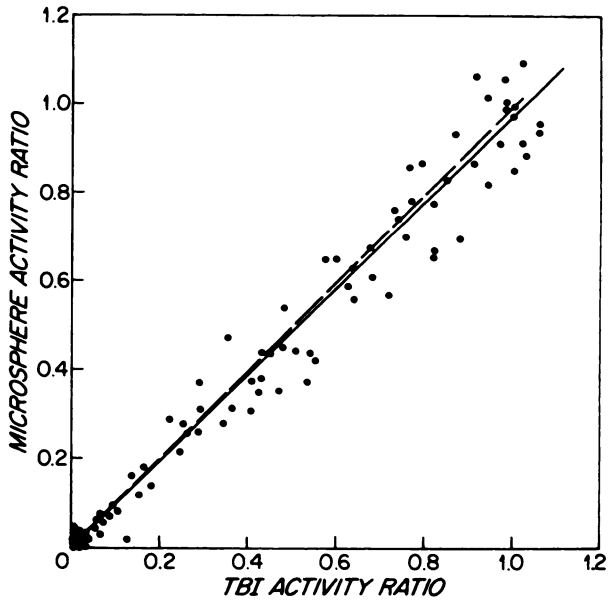

FIGURE 1
Correlation between myocardial concentration of [^{99m}Tc]TBI and regional myocardial blood flow during permanent coronary artery occlusion. Both [^{99m}Tc]TBI concentration and regional myocardial blood flow were normalized by values from distant normal myocardium. These normalized values for [^{99m}Tc]TBI and microsphere concentrations were called activity ratios. Each point represents one tissue sample analyzed for [^{99m}Tc]TBI concentration and regional myocardial blood flow. (– – –) = Line of identity; TBI = 0.97 RMBF − 0.01; r = 0.98; s.e.e. = 0.07

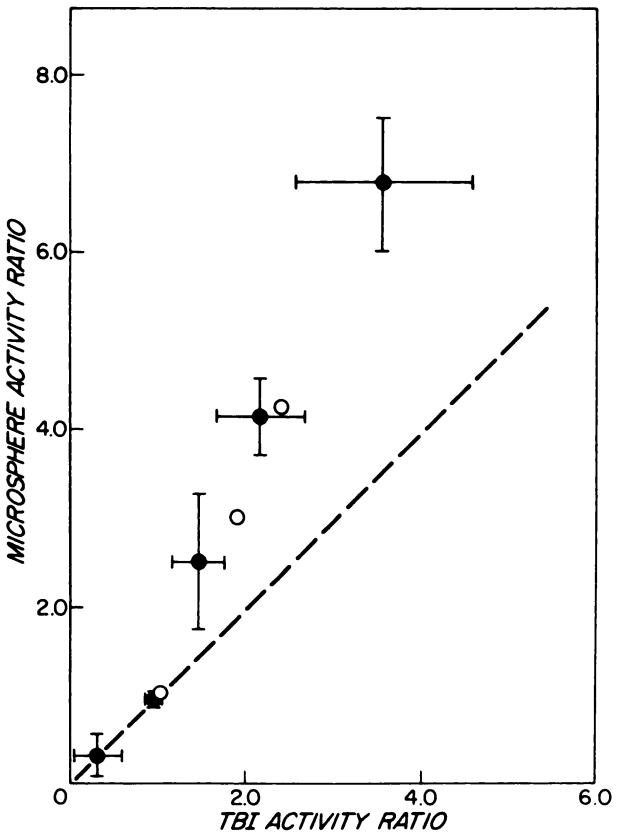

FIGURE 2
Relationship between [^{99m}Tc]TBI concentration and regional myocardial blood flow in normal myocardium and in zones of ischemia and transient hyperemia. Ischemic zones were obtained in three dogs following permanent occlusion of left anterior descending artery while zones of transient hyperemia were obtained from three other dogs after temporary occlusion of left anterior descending artery followed by reperfusion. In all cases, [^{99m}Tc]TBI concentration and regional myocardial blood flow were normalized by values obtained from distant normal myocardium. These normalized values for [^{99m}Tc]TBI and microsphere concentrations were called activity ratios. (O) = Relationship between normalized ^{201}Tl concentration and regional blood flow as reported by Strauss et al. (*7*). Bars represent 1 s.d. from mean

Protocol C

After the i.v. injection of [^{99m}Tc]TBI and ^{201}Tl 3 min prior to reperfusion, redistribution of the two tracers into ischemic myocardium was similar after both 10 and 30 min of reperfusion. The [^{99m}Tc]TBI concentration 10 min after reperfusion was 73.7 ± 2.2% (s.e.e.) of normal compared with 65.0 ± 2.1% of ^{201}Tl in tissue where blood flow was reduced to 40.7 ± 4.2% of normal prior to reperfusion (Fig. 3). The [^{99m}Tc]TBI concentration 30 min after reperfusion was 73.8% ± 1.1% of normal compared with 67.3 ± 1.4% for ^{201}Tl in tissue in which blood flow was reduced to 12.5 ± 3.3% of normal prior to reperfusion. The differences between the [^{99m}Tc]TBI concentration and blood flow were statistically significant with both 10 and 30 min of reperfusion ($p < 0.05$; Student's t-test for paired data).

Protocol D

After the coronary artery injection of [^{99m}Tc]TBI, the washout of the tracer from the myocardium was slow (Fig. 4). The myocardial concentration of tracer was 91.3 ± 6.7% (s.e.e.), 81.0 ± 6.4% and 78.5 ± 11.5% of the initial concentration 20–30, 40–60, and 120 min after injection, respectively.

DISCUSSION

Myocardial scintigraphy with ^{201}Tl has emerged as an important clinical tool for the assessment of myocardial perfusion in patients with coronary artery disease (*6*). Thallium-201 has a number of attractive properties. Its myocardial distribution is proportional to myocardial blood flow in normal and ischemic zones (*7*). In hyperemic zones, the relationship remains proportional. Since the myocardial distribution of ^{201}Tl undergoes redistribution in the face of transient ischemia, reversible ischemia can be distinguished from in-

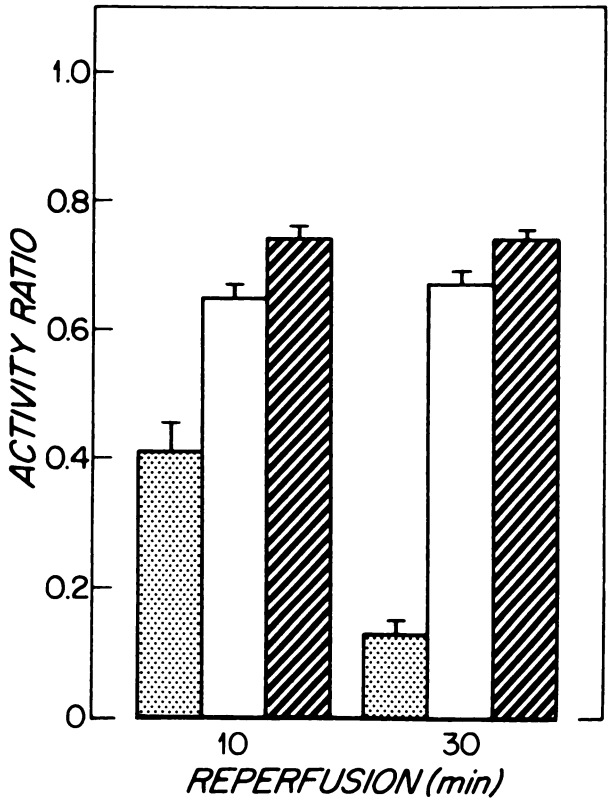

FIGURE 3
Redistribution of [^{99m}Tc]TBI compared with that of ^{201}Tl following temporary occlusion of left anterior descending artery. Microspheres were injected prior to reperfusion. [^{99m}Tc]TBI was injected intravenously 3 min prior to reperfusion and was measured after 10 or 30 min of reperfusion. All values were normalized to activity concentration in distant normal myocardium. These normalized values for [^{99m}Tc]TBI, ^{201}Tl and microsphere concentrations were called activity ratios. Error bars expressed as standard error of mean. (□) Microspheres; (▨) ^{201}Tl; (▨) ^{99m}TBI

farction and scar tissue following a single injection of the tracer (*8*).

Unfortunately, ^{201}Tl has physical characteristics that are far from optimal. Its physical half-life of 73 hr is long. Most of the photons associated with its decay are characteristic x-rays with low energies (69–83 keV) resulting in poor discrimination between scattered radiation and the primary photopeaks. These photons are highly attenuated in tissue resulting in poor sensitivity for deep tissues such as the posterior and inferior walls of the left ventricle. Since there is no generator system to produce ^{201}Tl, it must be commercially distributed shortly after production, increasing its cost and reducing its ready availability.

Technetium-99m offers substantial advantages over ^{201}Tl as a radionuclide for myocardial imaging. It is obtained from a commercially available generator system. It has a relatively short physical half-life (6 hr). It has a monoenergetic photopeak of 140 keV that is ideally suited to planar and tomographic imaging. For these reasons, considerable effort has gone into the development of ^{99m}Tc-labeled tracers that reflect myocardial perfusion. A number of these agents appeared promising based on their distribution in animals, but were disappointing when tested in humans because of poor myocardial uptake (*9–11*). More recently several of the hexakis (alkylisonitrile) technetium (I) cations have been developed and have shown marked cardiac uptake in a number of animal species (*1*). One of these, [^{99m}Tc]TBI, has been tested in humans (*2*). Its distribution in normal subjects and in patients with coronary artery disease suggests that its distribution is related to myocardial blood flow and that myocardial images of high quality can be obtained after lung clearance.

In this study, we have shown a very close correlation between [^{99m}Tc]TBI uptake and myocardial blood flow in zones of ischemia even when flow has been markedly reduced, as long as regional flow is unchanged from the time of injection to the time of imaging. In this respect, [^{99m}Tc]TBI and ^{201}Tl behave similarly.

In zones of transient hyperemia, [^{99m}Tc]TBI concentration increases with increasing flow, but at a rate that underestimates the degree of hyperemia. The underestimation of flow that we found for [^{99m}Tc]TBI is quite similar to the underestimation of flow that Strauss et al. found using ^{201}Tl as the tracer (*7*) in a similar model. The underestimation in uptake of both tracers may be due to either the short duration of the hyperemia or the failure of oxygen utilization to increase with increasing blood flow in this model. We also found a good deal of variability in the relationship between tracer concentration and blood flow from dog to dog perhaps because of variability in the duration of hyperemia.

The extent of [^{99m}Tc]TBI redistribution into zones of transient ischemia after an i.v. injection is similar to that of ^{201}Tl despite the very slow washout of [^{99m}Tc]TBI from the myocardium. Technetium-99m TBI redistribution is due to washin from the blood and probably results from slow lung clearance. Thallium-201 redistribution is due to both washin from the blood and washout of normal myocardium (*6*).

These observations have implications for the potential use of [^{99m}Tc]TBI as a myocardial imaging agent. With a steady state alteration in myocardial blood flow, the tracer distribution will reflect alterations in perfusion for many hours after injection and should, therefore, be an ideal imaging agent for screening patients with suspected myocardial infarction. On the other hand, we would predict that the rate of redistribution of the tracer into zones of transient ischemia would be rapid. While the model of transient hyperemia that we used probably overestimates the rate of redistribution found in patients with coronary artery disease, we found the redistribution rates of [^{99m}Tc]TBI and ^{201}Tl to be similar. We would therefore predict that the accuracy for the detection of ischemia would be reduced if we had to wait 30–60 min after injection for the lung activity to clear. To improve the accuracy with which transient ischemia is detected, the standard ^{201}Tl exercise myocardial scintigraphy protocol will have to be modified. The time between the injection of the tracer and the end of exercise should be increased to permit lung activity to clear as much as possible. This may be accomplished more easily by switching to pharmacological interventions such as dipyridamole than by increasing the length of maximal treadmill exercise. Alternatively, the various alkylisonitrile analogs have shown considerable variation in biodistribution and it should be possible to synthesize one with more rapid lung clearance.

The advantages of a ^{99m}Tc-labeled myocardial perfusion agent are overwhelming: better spatial resolution, lower radiation dose, lower tissue attentuation, more optimal tomographic imaging, and evaluation of wall motion with gated studies (*2*). Technetium-99m TBI has the further advantage of rapid redistribution so that, like ^{201}Tl, transient ischemia can be distinguished from infarction after a single injection. For these reasons it is well worth investigating modifications in the standard exercise protocol or modifications in the tracer so that it or one of its analogues can be used as a replacement for ^{201}Tl in the assessment of patients with known or suspected coronary artery disease.

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