Technetium-99m hexakis 2-methoxy-2-isobutyl isonitrile and Thallium-201 Extraction, Washout, and Retention at Varying Coronary Flow Rates in Rabbit Heart

Robert C. Marshall, MD, Edwin M. Leidholdt Jr., PhD, Dan-Ya Zhang, BA, and Charles A. Barnett, MD

The ability to accurately assess myocardial perfusion with thallium-201 suffers because its low 69-80-kev emission energy results in tissue photon attenuation and image degradation and its high radiation burden limits the amount of activity injected and image information density. In contrast, the 140-kev emission energy of technetium-99m is associated with less tissue photon attenuation and better image resolution, and its lower radiation burden allows a higher injected activity and improves imaging statistics. The superiority of 99mTc over 201Tl as an imaging isotope is the major reason for the great interest in the recently introduced 99mTc-labeled isonitrile compounds as potential alternatives to 201Tl as tracers of myocardial perfusion.1-6 Technetium-99m hexakis 2-methoxy-2-isobutylisonitrile (Tc-MIBI) is an isonitrile compound that has undergone preliminary clinical evaluation and appears to provide perfusion imaging results at least comparable to those of 201Tl.1

The tracer kinetic property that is fundamental to the measurement of myocardial perfusion with both 201Tl and Tc-MIBI is their tendency to accumulate in the myocardium so that tissue tracer content is proportional to the rate of coronary blood flow. The efficiency with which Tc-MIBI and 201Tl measure perfusion can be experimentally assessed by determining the extraction and retention of these two tracers over the range of flow rates encountered in

See p 1067
their clinical use. Maximally accurate evaluation of perfusion would occur under conditions in which Tc-MIBI and $^{201}$Tl extraction and retention are 100%, yielding a correlation between absolute tissue tracer content and absolute blood flow corresponding to the line of identity. Deviations from the line of identity will occur if either Tc-MIBI or $^{201}$Tl extraction or retention are less than 100%, and the accuracy of perfusion measurement will be reduced if flow itself has a direct effect on the extraction, retention, or clearance of either tracer. Previous studies have indicated that myocardial $^{201}$Tl uptake and retention are less than 100% and that flow has a direct effect on its clearance and possibly uptake. Initial evidence indicates that Tc-MIBI extraction and retention are also less than 100%. However, there is a paucity of data relating Tc-MIBI extraction, washout, and retention to blood flow. In addition, a simultaneous assessment of these three parameters for Tc-MIBI and $^{201}$Tl at varying coronary blood flow rates in the absence of tracer recirculation has not been undertaken.

Therefore, the purpose of the present study was to investigate and compare Tc-MIBI and $^{201}$Tl uptake and retention over a range of perfusion rates potentially relevant to their clinical use. To accomplish this goal, Tc-MIBI and $^{201}$Tl extraction, washout, and retention were simultaneously evaluated in 20 isolated, isovolumic, retrograde blood-perfused rabbit hearts at global left ventricular flow rates ranging from 0.50 to 3.50 ml/g wet wt min$^{-1}$. To more accurately evaluate previously extracted tracer retention and washout, tracer recirculation was excluded from the present experiments. The data obtained were used to 1) compare the extraction, washout, and retention of Tc-MIBI with $^{201}$Tl at varying flow rates; 2) assess and compare the effect of flow per se on Tc-MIBI and $^{201}$Tl extraction, washout, and retention; and 3) evaluate and compare the abilities of Tc-MIBI and $^{201}$Tl to measure myocardial blood flow under the single-pass conditions used in the present study.

**Methods**

**Experimental Preparation**

Preparation of isovolumically beating, retrograde blood-perfused rabbit hearts was similar to that previously reported. Briefly, after administration of 10 mg Na heparin and 200 mg Na pentobarbital via an ear vein, hearts from nonfasted, male New Zealand White rabbits (2.0–2.5 kg) were excised through a median sternotomy and arrested in ice-cold saline. The aorta was rapidly cannulated to allow retrograde perfusion with red blood cell-containing perfusate. Flow was held constant at 2.0 ml/g wet wt min$^{-1}$ with a Minipuls 2 peristaltic pump (Gilson Co., Inc., Worthington, Ohio). The red blood cell-containing perfusate was not recirculated.

An apical drain was inserted through the left atrium into the left ventricle to allow drainage of fluid from the Thebesian circulation. After the atrio-

ventricular node was crushed to allow controlled stimulation, a fluid-filled latex balloon connected to a Gould-Statham P23ID pressure transducer (Gould, Inc., Oxnard, Calif.) was inserted into the left ventricle via the left atrium and mitral valve. A coronary venous sampling catheter and needle thermistor (Bailey Instrument Co., Inc., Saddlebrook, N.J.) were inserted into the right ventricle via the right atrium and tricuspid valve. The venae cavae and pulmonary artery were then ligated so all coronary venous drainage flowed out the sampling catheter.

Hearts were perfused with a modified Tyrode’s solution containing 20% oxygenated bovine red blood cells and 17–18 gm/l bovine serum albumin (free fatty acid free, Sigma Chemical Co., St. Louis, Mo.). The specific electrolyte concentrations were (mM) NaCl 110.0, CaCl$_2$ 2.5, KCl 6.0, MgCl$_2$ 1.0, NaH$_2$PO$_4$ 0.435, and NaHCO$_3$ 28.0. Bovine serum albumin was prepared by overnight dialysis at 4°C against a large volume of buffer and filtration through a 0.8-μm Millipore filter. Dextrose (11 mM) and Na acetate (2.0 mM) were provided to ensure adequate substrate availability to the myocardium. Bovine red blood cells were oxygenated by washing five times in ice-cold perfusate that had been equilibrated with 100% oxygen. Final perfusate pH was adjusted to between 7.3 and 7.4 by equilibrating the buffer in which red blood cells were suspended for myocardial perfusion with 98% O$_2$-2% CO$_2$. An in-line Swank transfusion filter (13 μm exclusion, model IL200, Pioneer Viggo, Inc., Beaverton, Ore.) was used to filter red blood cell aggregates.

After the preparation was complete, the left ventricular balloon was inflated until end-diastolic pressure was 8 mm Hg. Stimulating electrodes from a Grass SD 44 stimulator (Quincy, Mass.) were placed against the left and right ventricles, and 4-V, 4-msec stimuli were delivered at a rate of 180 min$^{-1}$. Left ventricular end-diastolic pressure and stimulus rate were invariant throughout each experiment. Similarly, temperature was maintained between 36° and 38°C with a water-jacketed perfuse-heating coil and heart chamber.

**Radiopharmaceuticals**

Tc-MIBI was prepared from a kit supplied by E.I. du Pont de Nemours and Co. Biomedical Products, No. Billerica, Mass. Approximately 5 mCi $^{99m}$Tc pertechnetate was added to the kit, and the isotope-containing vial was placed in boiling water for 15 minutes. After the vial had cooled, radiochemical purity was checked by chromatographic analysis (SEP-PAK). The radiochemical purity was 95% or greater in all cases. $^{201}$Tl and iodine-125 albumin were also obtained from E.I. du Pont.

**Experimental Protocol**

An equilibration period of 15 minutes preceded all experimental interventions. A preparation was accepted as suitable for study if it developed at least 80 mm Hg pressure (peak systolic minus end-
diastolic) at an end-diastolic pressure of 8 mm Hg and if end-diastolic and end-systolic pressures were stable over the equilibration period. After equilibration, myocardial perfusion was changed to the experimental flow rate and subsequently held constant by the perfusion pump so each heart was studied at only one perfusion rate. The particular flow rate evaluated in individual experiments was predetermined from an alternating schedule of stepwise decreases and increases in perfusion between 0.5 and 3.5 ml/g wet wt min⁻¹.

After a 5-minute stabilization period at the experimental flow rate, a mixed isotope bolus (0.2 ml) consisting of ¹²⁵I-albumin (10–12 μCi), Tc-MIBI (5–6 μCi), and ²⁰¹TI (5–6 μCi) was injected just above the aortic cannula. Rapid venous sampling from the right ventricular cannula (initially, 3–10 sec/sample depending on flow rate; subsequently, 15 sec/sample) commenced with radioisotope introduction and was continued uninterrupted for 25–30 samples. Subsequent samples were acquired at progressively greater intervals (30 sec/sample to 15 min/sample) so a total of approximately 50 venous samples were obtained over a period of 40–60 minutes after isotope injection.

**Data Acquisition and Analysis**

Venous samples and multiple aliquots (n=10) of a diluted solution containing the mixed isotope bolus were counted on an automated gamma counter (Beckman Instruments, Fullerton, Calif.). Correction for cross-contamination between nuclides was performed as follows: 1) ⁹⁹ᵐTc was counted with a window centered around its 140-kev photopeak on the same day of the experiment, 2) ²⁰¹TI was counted 7 days later (28×t½ for ⁹⁹ᵐTc) with a window centered around its 167-kev photopeak; and 3) ¹²⁵I activity was assessed 15 days after the experiment with a window encompassing its 28–31-kev X-ray photopeak and 60-kev sum peak. Crossover corrections were performed with coefficients determined from pure radionuclides and assuming that ²⁰¹TI activity was uncontaminated. Counting errors related to dead-time losses and volume effects were minimized to 2% or less. All count rate data were decay-corrected to the time of injection. Mean recovery of ¹²⁵I-albumin for the 20 experiments was 95±3% (range, 90–102%).

Myocardial extraction, washout, and retention of Tc-MIBI and ²⁰¹TI were estimated from the directly measured venous outflow activity expressed as the fraction of injected activity appearing per second [h(t)], or fractional venous appearance rate, and calculated as disintegrations per minute (dpm) [(dpm/ml sample/dpm injected)×flow (ml/sec)]. Instantaneous tracer extraction, E(t), was computed relative to the vascular reference tracer as:

\[ E(t) = \frac{[h(t) - h_d(t)]}{h(t)} \]

where \( h_d(t) \) is the directly measured fractional venous appearance rate of ¹²⁵I-albumin, and \( h_d(t) \) is the directly measured fractional venous appearance rate of either Tc-MIBI or ²⁰¹TI. Peak Tc-MIBI or ²⁰¹TI extraction, \( E_{peak} \), was taken as the maximum instantaneous extraction value for each tracer. Net extraction, \( E_{net}(t) \), a measure of net tracer retention, was calculated from the outflow curves as the following time integral:

\[ E_{net}(t) = \frac{\int_{0}^{t} [h_d(\lambda) - h_d(\lambda)] d\lambda}{\int_{0}^{t} h_d(\lambda) d\lambda} \]

where \( \lambda \) is a variable of integration. Net tissue tracer uptake, a function of tracer delivery as well as \( E_{net}(t) \), was determined from the product of flow and \( E_{net}(t) \) [F×E_{net}(t)]. Fractional escape rate, FER(t), a measure of washout, was computed as the ratio of the tracer fractional venous appearance rate h(t) and the tracer residue function (a second measure of net tracer retention):

\[ FER(t) = \frac{h_d(t)}{R_d(t)} \]

where

\[ R_d(t) = 1 - \int_{0}^{t} h_d(\lambda) d\lambda \]

These computations have been used by other investigators¹⁵ and are valid in the presence of tracer back-diffusion.*

Because the presently used venous sampling technique measures whole heart and collection-tubing Tc-MIBI and ²⁰¹TI transit, extraction, washout, and retention, the relation between technetium and thallium retention calculated from venous samples and directly measured left ventricular tracer content was studied in 10 additional hearts at steady-state flow rates ranging from 0.68 to 2.8 ml/g wet wt min⁻¹ (mean flow, 1.66±0.80 ml/g wet wt min⁻¹). These experiments were identical to those described above except that flow was stopped and the heart was immediately cut off of the perfusion cannula at 15–16 minutes after tracer introduction. Right ventricular, atrial, and connective tissues were then trimmed away, and the left ventricle was rinsed, blotted dry, and cut into 12 segments of 0.2–0.5 g each. Tissue sample counting was identical to that for venous samples. Total thallium and technetium activities retained in the left ventricle were computed from the 12 ventricular segments, expressed as a fraction of total injected isotope activity, and compared with the corresponding \( E_{net}(t) \) values calculated from the simultaneously acquired venous samples.

*It should be noted that the tracer residue function (R(t)) and net extraction (E_{net}(t)) become equal when all of the reference tracer (¹²⁵I-albumin) has been recovered. Functionally, \( E_{net}(t) \) is the same as R(t) corrected for unextracted tracer remaining in the vascular space.
For the 10 hearts, the ratio of total thallium to total technetium tissue activity determined directly from left ventricular tissue correlated well with the corresponding $E_{net}(t)$ value ratios computed from venous samples ($r=0.84$; mean tissue $^{201}$Tl/$^{99m}$Tc, 0.67±0.32; range, 0.33–1.17; mean calculated $E_{net}^{201}$Tl/$^{99m}$Tc, 0.73±0.36; range, 0.33–1.44). Similarly, absolute values for directly measured fractional tissue tracer content correlated well with calculated $E_{net}^{201}$Tl values for technetium ($r=0.88$) and thallium ($r=0.84$). However, presumably at least in part reflecting right ventricular and atrial tracer extraction, the mean values for $E_{net}(t)$ computed from venous samples exceeded the mean values for fractional tissue content for both tracers ($^{99m}$Tc: mean $E_{net}$ 0.27±0.06; mean fractional tissue content, 0.21±0.06; $p<0.01$; $^{201}$Tl: mean $E_{net}$ 0.19±0.05; mean fractional tissue content, 0.14±0.06; $p<0.01$). These data indicate that any detected changes in Tc-MIBI and $^{201}$Tl retention related to flow rate and relative Tc-MIBI compared with $^{201}$Tl retention computed from venous samples primarily reflect left ventricular tracer activity and therefore support the venous sampling technique used in the present study.

Statistics

Values are given as mean±SD. All statistical calculations required for regression analysis were performed with Statgraphics statistical graphics system, Statistical Graphics Corp., Rockville, Md. Data used for regression analysis were fit to linear, exponential, multiplicative, and reciprocal models with estimated regression coefficients derived by the least-squares method. Comparison of Tc-MIBI and $^{201}$Tl fractional escape rates (Figure 3) was performed with the two-tailed Mann-Whitney rank-sum test. All other statistical comparisons were undertaken with Student’s t test for paired or unpaired data. A p value of less than 0.05 was considered statistically significant. All experiments conformed to guidelines established by the American Physiological Society.

Results

Experimental Relations Between $h(t)$, $E(t)$, and $E_{net}(t)$

Figure 1 provides examples of Tc-MIBI, $^{201}$Tl, and $^{125}$I-albumin fractional venous appearance rates [$h(t)$] and corresponding Tc-MIBI and $^{201}$Tl instantaneous extraction [$E(t)$] and net extraction [$E_{net}(t)$] values obtained during a single transit of the mixed isotope bolus through the heart. The results from one heart evaluated at a coronary flow rate of 2.0 ml/g wet wt min$^{-1}$ are shown in the left column (Figures 1A, 1C, and 1E), and the results from a second heart studied at 0.5 ml/g wet wt min$^{-1}$ are shown in the right column (Figures 1B, 1D, and 1F). In both experiments, the initial $h(t)$s of Tc-MIBI and $^{201}$Tl (Figures 1A and 1B) are less than the vascular tracer, $^{125}$I-albumin, reflecting early myocardial extraction of the two flow tracers. After approximately 2 minutes, $h(t)$s of Tc-MIBI and $^{201}$Tl exceed that of $^{125}$I-albumin, indicating back-diffusion of previously extracted tracer. In Figures 1C and 1D, the positive $E(t)$ values for Tc-MIBI and $^{201}$Tl are seen to correspond in time to the initial portion of the indicator dilution curves during which the $h(t)$s of the two flow tracers are less than that of the vascular tracer [negative $E(t)$ values are not shown]. In Figures 1E and 1F, values for Tc-MIBI and $^{201}$Tl $E_{net}(t)$ are highest soon after tracer injection with maximum $E_{net}(t)$ [$E_{net,max}$ occurring at the time of peak $E(t)$]. Subsequently, Tc-MIBI and $^{201}$Tl $E_{net}(t)$ decline for the remainder of the experiment. These patterns just described for Tc-MIBI and $^{201}$Tl $h(t)$, $E(t)$, and $E_{net}(t)$ are representative of those observed in all experiments.

Flow Compared With Peak Instantaneous Extraction

Figure 2 illustrates the effect of altered flow on Tc-MIBI and $^{201}$Tl peak $E(t)$ values ($E_{peak}$) for all 20 hearts. The $E_{peak}$ of each tracer declined as flow increased ($p<0.001$). For $^{201}$Tl, $E_{peak}$ declined from 0.91 at the lowest flow rate to 0.68 at the highest flow rate, whereas Tc-MIBI declined from 0.71 to 0.37 at comparable flows. The decline in $E_{peak}$ with increasing flow was significantly greater for Tc-MIBI than for $^{201}$Tl (mean change in $E_{peak}$ for Tc-MIBI, 0.24±0.05; mean change in $E_{peak}$ for $^{201}$Tl, 0.14±0.03; $p<0.05$) when the $E_{peak}$ values obtained in the five hearts studied at the lowest flow rates (mean flow, 0.68±0.19 ml/g wet wt min$^{-1}$) were compared with the mean $E_{peak}$ value determined from the five hearts with the highest flow rates (mean flow, 2.78±0.58 ml/g wet wt min$^{-1}$). In addition, the best-fit relations describing the effect of flow on $E_{peak}$ were quantitatively different for Tc-MIBI (reciprocal) than for $^{201}$Tl (linear). These data were interpreted to indicate that increasing the rate of coronary blood flow decreases the $E_{peak}$ of both tracers and that the $E_{peak}$ of Tc-MIBI is lower and demonstrates a more pronounced decline with increased flow than $^{201}$Tl $E_{peak}$.

Tc-MIBI and $^{201}$Tl Washout

To assess the possibility of time-variable Tc-MIBI and $^{201}$Tl washout, the mean fractional escape rates for both tracers were plotted as functions of time for the five hearts studied at the highest and lowest flow rates (see above) (Figure 3). For both sets of hearts, evaluation of tracer washout was initiated when 90% of recoverable vascular tracer ($^{125}$I-albumin) had been collected (33±8 and 81±15 seconds after isotope introduction for high- and low-flow hearts, respectively). Therefore, the rapid decline in fractional escape rate seen in all curves between 1 and 5 minutes probably reflects a diminishing contribution to washout of tracer from vascular and interstitial spaces. Between 5 and 40 minutes, the decline in Tc-MIBI and $^{201}$Tl washout, although significant ($p<0.001$), was less steep than that observed before 5 minutes in both sets of experiments. However, in hearts studied during high flow, the values for $^{201}$Tl fractional escape rate between 5 and 20 minutes were higher ($p<0.05$) and
exhibited a greater reduction with time than those for Tc-MIBI (p=NS for 201Tl compared with Tc-MIBI 30- and 40-minute fractional escape rate values). In contrast, in hearts studied at low-flow rates, the early rapid washout and marked time-related reduction in thallium fractional escape rates were not observed, although the fractional escape rates for 201Tl were still significantly greater than Tc-MIBI at all times evaluated (p<0.05). These data indicate that 1) 201Tl washout is faster than Tc-MIBI washout from 5 to 20 minutes after isotope injection in high-flow experiments and for as long as 40 minutes in low-flow hearts, and 2) there is an early rapid washout of 201Tl that declines with time during high-flow experiments not observed for 201Tl during low flow or Tc-MIBI at either flow rate.

The effect of altered flow per se on Tc-MIBI and 201Tl fractional escape rates was analyzed at 10 and 30 minutes for all 20 hearts (data not shown). At 10 minutes, the fractional escape rates of both tracers increased as flow increased (p<0.001). In addition, the increase in fractional escape rate values with
increasing flow rates was more profound for 201Tl than for Tc-MIBI as evidenced by a mean increase of 4.55±0.62 compared with 1.22±0.71×10−3 sec−1, respectively (p<0.05) when the five high-flow and five low-flow hearts were compared. At 30 minutes, the effect of flow on Tc-MIBI fractional escape rate was similar to that observed at 10 minutes. However, variations in blood flow caused no discernible change in 201Tl fractional escape rate at 30 minutes. These data show that 201Tl washout is initially strongly influenced by flow rate, whereas Tc-MIBI washout demonstrates a relatively modest flow effect.

**Tc-MIBI and 201Tl Net Extraction**

Figure 4 illustrates the mean myocardial Fnet(t) (or retention) of Tc-MIBI and 201Tl plotted as a function of time for the five hearts studied at the highest (Figure 4A) and lowest (Figure 4B) flow rates. As predicted by relative Tc-MIBI and 201Tl extraction and washout rate, maximal 201Tl Fnet(t) is significantly greater (p<0.001) and declines at a faster rate (p<0.001) than Tc-MIBI extraction at both flow rates. Similarly, reflecting the effect of flow on TcMIBI and 201Tl extraction and washout, increasing myocardial perfusion rate decreased the maximum Fnet(t) of both tracers [Tc-MIBI Fnet max: 0.53±0.045 compared with 0.34±0.017; 201Tl Fnet max: 0.82±0.053 compared with 0.61±0.035 (low-flow compared with high-flow hearts, respectively); p<0.001 for both comparisons]. In addition, increased flow accelerated the rate of decline in tracer retention [Tc-MIBI mean decline/min: 0.2±0.1% compared with 1.0±0.2%; 201Tl mean decline/min: 1.4±0.1% compared with 3.0±0.2% (low-flow compared with high-flow hearts, respectively); p<0.001 for both comparisons]. These latter data clearly indicate a direct effect of perfusion rate on myocardial retention of both Tc-MIBI and 201Tl.

**Flow Compared With Relative Net Tc-MIBI and 201Tl Uptake**

The effect of flow-related variability in tissue tracer retention on the capacities of Tc-MIBI and 201Tl to measure myocardial blood flow was assessed by correlating net tissue tracer uptake [F×Fnet(t)] and flow over the entire range of perfusion rates evaluated in this study (Figure 5). Maximum net tissue tracer uptake was computed with the maximum value for Fnet(t) (F×Fnet max). The decline in net tissue tracer uptake with time was evaluated by also computing similar values at 10 (F×Fnet10) and 30 minutes (F×Fnet30) after tracer injection. Data for net uptake
uptake and flow were nonlinear (multiplicative) for both Tc-MIBI and \(^{201}\text{Tl}\).

At 10 (Figures 5C and 5D) and 30 minutes (Figures 5E and 5F) after tracer introduction, relative Tc-MIBI and \(^{201}\text{Tl}\) uptake progressively underestimate changes in flow rate over the entire range of perfusion rates evaluated. Similar to that observed immediately after tracer injection, the underestimation of flow is more serious at high coronary flow rates for both Tc-MIBI and \(^{201}\text{Tl}\) at 10 and 30 minutes. However, in contrast to maximal tracer uptake, the superiority of \(^{201}\text{Tl}\) as a perfusion tracer is no longer apparent at the two later time periods \((p=\text{NS} \text{ for both comparisons})\). In summary, these data indicate that \(^{201}\text{Tl}\) is initially a better tracer of myocardial perfusion than Tc-MIBI and that the superiority of \(^{201}\text{Tl}\) as a flow indicator disappears by 10 minutes.

**Discussion**

In this investigation, the extraction, washout, and retention of Tc-MIBI and \(^{201}\text{Tl}\) were evaluated and compared at coronary flow rates ranging from 0.5 to 3.5 ml/g wet wt min\(^{-1}\) in the absence of tracer recirculation. The results of this study reveal that 1) Tc-MIBI is both less well extracted and has a slower rate of washout than \(^{201}\text{Tl}\); 2) increasing the rate of coronary blood flow diminishes extraction, increases washout, and decreases the retention of both tracers; and 3) the reduction in Tc-MIBI extraction with increased perfusion is more pronounced than \(^{201}\text{Tl}\), whereas the increase in initial \(^{201}\text{Tl}\) washout is more affected by flow rate than Tc-MIBI washout. Reflecting its higher, less flow-sensitive extraction, relative maximal myocardial thallium uptake correlates more closely with perfusion rate than relative maximal Tc-MIBI uptake. However, the early superiority of \(^{201}\text{Tl}\) is lost with time, presumably due to its more rapid washout, particularly under high-flow conditions. The results of this study were interpreted to indicate that although there are important quantitative differences between Tc-MIBI and \(^{201}\text{Tl}\) extraction, washout, and retention, the present results support continued evaluation of Tc-MIBI as a potential alternative to \(^{201}\text{Tl}\) as a tracer of myocardial perfusion. On the other hand, the incomplete, flow-dependent extraction, flow-dependent washout, and nonlinear relation between net uptake and flow observed for Tc-MIBI do not support its consideration as a "molecular microsphere," and similar observations for \(^{201}\text{Tl}\) indicate that both Tc-MIBI and \(^{201}\text{Tl}\) have important limitations as perfusion indicators.

**Relation of Present Results to Clinical Imaging Studies**

The present results were obtained under conditions in which a range of coronary blood flow rates were initially produced with individual flow rates subsequently held constant during data acquisition after Tc-MIBI and \(^{201}\text{Tl}\) introduction. These experimental conditions appear comparable to clinical values.

---

**Figure 4.** Plot of mean myocardial technetium-99m hexakis 2-methoxy-2-isobutyl isonitrile (Tc-MIBI) and thallium-201 retention \(\left[E_{\text{net}}(t)\right]\) curves determined from five high-flow (panel A) and five low-flow (panel B) hearts. Average SDs are (percent of mean value): Tc-MIBI high-flow, 17\% (range, 10-29\%); Tc-MIBI low-flow, 9\% (range, 5-32\%); \(^{201}\text{Tl}\) high-flow, 9\% (range, 4-18\%); \(^{201}\text{Tl}\) low-flow, 10\% (range, 5-29\%). Solid lines represent visually determined best-fit relations.
FIGURE 5. Plots of relations between relative net technetium-99m hexakis 2-methoxy-2-isobutyl isonitrile (Tc-MIBI) (left) and thallium-201 (right) uptake and flow immediately after tracer injection (panels A and B) and 10 (panels C and D) and 30 minutes (panels E and F) later. Initial net tracer uptake was computed with maximal value for net extraction \( [E_{net}(t)]_0 \) \( [E_{net max}] \). Values are given as relative net tissue tracer uptake compared with that obtained at a flow of 1.0 ml/g wet wt min \(^{-1} \). Reference values at 1.0 ml/g wet wt min \(^{-1} \) were computed as average of 10 hearts contained in flow range of 0.67 to 1.36 ml/g wet wt min \(^{-1} \). Relative Tc-MIBI and \(^{201}\text{TI} \) uptake were calculated by dividing values from individual hearts by corresponding reference values. Data points are results from individual hearts, and solid curves represent best-fit relations determined from given equations. Straight dashed lines are lines of identity.

studies performed after dipyridamole infusion or to those obtained in the basal state in the presence of heterogeneous blood flow.\(^{16}\) Although there is clearly a period of heterogeneous flow at the end of exercise and immediately after exercise, extrapolation of the present results to exercise and rest studies in patients with coronary artery disease is made difficult because of the development of non-steady-state, postexercise flow and the potential for homogeneous resting flow.

Under clinical conditions in which flow is maintained heterogeneous, the marked reduction in tissue Tc-MIBI and \(^{201}\text{TI} \) \( E_{net}(t) \) with time observed here
(Figure 4) will be attenuated after intravenous tracer administration because of the off-setting effect of continued recirculating tracer extraction. Because the decline in tissue tracer $E_{net}(t)$ is responsible for the time-related deterioration in the relations between relative net Tc-MIBI and $^{201}$TI uptake and perfusion rates (Figure 5), a potential result of recirculated tracer extraction would be to prolong the time period over which relative net tissue tracer uptake more accurately reflects blood flow. However, despite the tendency for recirculating tracer extraction to retard the time-dependent decrease in the value of $E_{net}(t)$, it will not halt the decline entirely because of washout of previously extracted Tc-MIBI and $^{201}$TI. As a result, the relations between flow and maximal net uptake ($F \times E_{net \max}$) for Tc-MIBI and $^{201}$TI represent best-possible or limiting conditions that might persist only immediately after tracer injection. Because of its faster, more flow-dependent rate of washout, the relation between flow and $^{201}$TI net uptake will deteriorate more rapidly with time than that for Tc-MIBI. Therefore, extrapolation of the present single-pass data to potentially comparable clinical situations suggests that 1) relative to the present findings, continued extraction of recirculating tracer will tend to attenuate the decrease in Tc-MIBI and $^{201}$TI retention and prolong the time period over which relative tissue tracer net uptake accurately reflects blood flow; 2) $^{201}$TI will be initially superior to Tc-MIBI as a perfusion indicator; and 3) this initial superiority of $^{201}$TI might disappear shortly after tracer injection. Because of the complexities of clinical imaging, including variable $^{201}$TI clearance and redistribution rates and uncertainties about potential factors that could affect Tc-MIBI clearance, it is not possible to predict the exact time at which $^{201}$TI will lose its advantage over Tc-MIBI as a perfusion indicator in individual patients.

Under the present experimental conditions, initial Tc-MIBI and $^{201}$TI extractions and their subsequent fractional escape rates provide qualitative estimates of relative forward and backward flux rates between blood and myocardium. Comparing Tc-MIBI with $^{201}$TI, the higher initial extraction for thallium is consistent with a lower resistance for forward flux into the myocardium, presumably due to a higher capillary and cell membrane permeability for $^{201}$TI compared with Tc-MIBI. Similarly, the higher fractional escape rate for thallium is consistent with a faster rate of backward flux out of the myocardium and suggests that $^{201}$TI is less efficiently trapped in the myocardium than Tc-MIBI. The faster rates for thallium forward and backward flux suggested by the present data are in agreement with a recent report using cultured myocardial cells \(^3\) and could indicate that tissue and blood thallium content exchange at a faster rate than Tc-MIBI. If these data acquired in vitro are applicable to the clinical situation, the relatively rapid interchange between blood and myocardium thallium content could contribute to the apparent steady-state tissue:plasma concentration gradient reflected in the equivalent blood and myocardial thallium clearance rates observed after systemic tracer administration.\(^5\) To the extent that the presence of a uniform steady-state tissue:plasma concentration gradient plays a role in the delayed tendency for thallium to develop a homogeneous concentration in viable myocardium,\(^17,18\) the rapid interchange between blood and myocardial thallium content might also contribute to the process of redistribution. In contrast, the slower myocardial-blood exchange rates for Tc-MIBI could retard the development of a homogeneous, steady-state tissue:plasma concentration gradient and thereby limit the tendency for this tracer to redistribute.

**Comparison of Present Results to Previous $^{201}$TI Studies**

The present mean $E_{peak}$ value for $^{201}$TI for all 20 experiments (0.83±0.06) is in the range reported by others.\(^7,10\) Previous studies assessing the effect of flow on $^{201}$TI extraction have noted that while extraction is minimally affected by flow when flow and work load change concordantly,\(^7,10,19\) flow and $E_{peak}$ are inversely related when perfusion is increased pharmacologically in excess of myocardial work load and metabolic needs.\(^7\) Under the present experimental conditions, the increase in flow exceeds the increase in isovolumic contractile function,\(^13\) which could account for the inverse relation between flow and extraction observed here. Similar to the present results, most\(^9,10\) but not all\(^20\) previous investigations reported an increase in $^{201}$TI washout and a reduction in $^{201}$TI retention with increased myocardial perfusion rates. Therefore, the present results for $^{201}$TI appear to be in substantive agreement with those reported previously.

**Comparison of Present Results to Previous Tc-MIBI Studies**

In a recently published report, Leppo and Meerdink\(^6\) evaluated Tc-MIBI and $^{201}$TI $E(t)$ and $E_{net}(t)$ in isolated blood-perfused rabbit hearts at flow rates comparable to those used in the present study. In addition, they computed the rates of trans-capillary and cell membrane tracer transport with computer-based modeling techniques. Similar to the present results, the peak instantaneous extractions of both tracers declined as flow was increased. Possibly reflecting differences in perfusion technique [e.g., nonrecirculating (present study) compared with recirculating (previous study)], the absolute values for $E_{peak}$ of both $^{201}$TI (0.73±0.10) and Tc-MIBI (0.39±0.09) were somewhat lower than those observed in the present investigation (0.83±0.06 and 0.55±0.10, respectively). Based on their model analysis, Leppo and Meerdink reported that capillary permeability was greater for $^{201}$TI than for Tc-MIBI, whereas the reverse was true at the cell membrane, an observation that they interpreted to be consistent with differences in the transport mechanisms of these two tracers. The quantitative differences in Tc-MIBI and $^{201}$TI flow-dependent extraction and washout
observed in the present study are similarly consistent with the possibility that distinct mechanisms could be involved in the transport of these two perfusion indicators between blood and myocardium.

In two investigations evaluating the in vivo relation between Tc-MIBI tissue content and blood flow during myocardial ischemia (i.e., flow rates, \( \leq 1.2 \) ml/g wet wt \( \text{min}^{-1} \)), relative changes in regional Tc-MIBI activity correlated linearly with blood flow. Consistent with the present observation that relative net Tc-MIBI uptake underestimated changes in flow rate, one of these studies noted a similar discrepancy between relative Tc-MIBI tissue content and simultaneously determined microsphere estimates of blood flow. One major discrepancy between the present data and data acquired in vivo is the difference in tissue Tc-MIBI retention time. In the study by Okada et al.,

A fractional clearance of 0.11 over 4 hours was noted in dogs, which corresponds to a \( t_{1/2} \) of 15–20 hours. When the present data for Tc-MIBI were replotted semilogarithmically to estimate tissue \( t_{1/2} \) values of 68±26 and 129±46 minutes were obtained for the high-flow and five low-flow hearts, respectively. These latter values are a full order of magnitude less than that observed in vivo. Because the longer \( t_{1/2} \) was recorded in the presence of tracer recirculation, it is possible that continued extraction of recirculating tracer might have prolonged in vivo myocardial Tc-MIBI retention time relative to that presently observed in vitro. A second disparity between the present data and data acquired in vivo is the presence of a modest flow-related increase in Tc-MIBI washout under the present single-pass conditions and the lack of a flow effect on Tc-MIBI clearance after systemic tracer administration. One possible explanation for this latter disparity is that increased delivery and extraction of recirculating tracer could mask an effect of accelerated flow on in vivo tracer clearance. Because myocardial uptake of recirculated tracer and altered flow rates could have an important effect on Tc-MIBI retention and clearance, additional investigations are needed to clarify these apparent conflicts between data acquired in vitro under single-pass conditions and data acquired in vivo after systemic tracer administration.

Acknowledgments

We thank Dr. Chester Mathis for his thoughtful review of this manuscript. We also acknowledge the expert technical assistance of Ms. Jill Perkins, the proficient secretarial assistance of Ms. Claudette Lamothe, and the excellent illustration and photography work of Mr. H. Morgan.

References


Key Words • technetium-99m • myocardial kinetics • thallium-201 • perfusion
Technetium-99m hexakis 2-methoxy-2-isobutyl isonitrile and thallium-201 extraction, washout, and retention at varying coronary flow rates in rabbit heart.
R C Marshall, E M Leidholdt, Jr, D Y Zhang and C A Barnett

Circulation. 1990;82:998-1007
doi: 10.1161/01.CIR.82.3.998

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/82/3/998