Quantification of Area at Risk During Coronary Occlusion and Degree of Myocardial Salvage After Reperfusion With Technetium-99m Methoxyisobutyl Isonitrile

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Serial myocardial imaging with technetium-99m methoxyisobutyl isonitrile (99mTc-MIBI) has been proposed for evaluating myocardial salvage after reperfusion. To define 99mTc-MIBI uptake before and after reperfusion, 17 open-chest dogs underwent 3 hours of left anterior descending artery occlusion and 3 hours of reperfusion. 99mTc-MIBI was injected during occlusion (group 1) or after 90 minutes of reperfusion (group 2). Myocardial 99mTc-MIBI activity was correlated with microsphere flow during occlusion and reperfusion. Anatomic risk area and infarct area were defined by postmortem vital staining and correlated with the perfusion defects defined by analysis of 99mTc-MIBI macroautoradiographs and gamma camera images of myocardial slices. The left ventricle was divided into 96 segments for gamma well counting. Flow and 99mTc-MIBI activity were normalized to nonischemic values. Myocardial segments were grouped, based on occlusion flow, into zones: severely ischemic (≤30% nonischemic), moderately ischemic (>30%, ≤60% nonischemic), mildly ischemic (>60%, ≤90% nonischemic), and nonischemic (>90%, ≤120% nonischemic). Among dogs injected with 99mTc-MIBI during coronary occlusion (group 1), myocardial 99mTc-MIBI activity correlated linearly with occlusion flow for both endocardial (r=0.91) and transmural (r=0.91) segments. The risk area defined by 99mTc-MIBI autoradiography (group 1) correlated with the postmortem risk area (r=0.94) but was 29% smaller than the anatomic risk area (p=0.03), reflecting the contribution of collateral flow. Among dogs injected with 99mTc-MIBI after reperfusion (group 2), myocardial 99mTc-MIBI did not correlate with reperfusion flow in either endocardial or transmural segments. Among group 2 dogs, myocardial 99mTc-MIBI activity was significantly less than reperfusion flow at the time of injection in the severely ischemic (25±5% versus 74±24% nonischemic, p=0.002), moderately ischemic (54±12% versus 96±15% nonischemic, p=0.001), and mildly ischemic (84±6% versus 93±3% nonischemic, p=0.002) zones. The defect area defined by 99mTc-MIBI autoradiography (group 2) correlated very closely with the postmortem infarct area (p=0.98). Thus, the myocardial uptake of 99mTc-MIBI during coronary occlusion correlates with occlusion flow and reflects the "area at risk." When 99mTc-MIBI was given after 90 minutes of reperfusion following 3 hours of coronary occlusion, the myocardial activity was significantly reduced compared with reperfusion flow in both necrotic and perinecrotic regions, reflecting myocardial viability more than the degree of reperfusion. (Circulation 1990;82:1424–1437)

The current management of myocardial infarction focuses on the application of acute interventional reperfusion techniques to reduce myocardial necrosis.1 During myocardial infarction, the extent of myocardial necrosis is determined by the "area at risk," collateral flow, and the duration of coronary occlusion.2 A noninvasive imaging technique that could 1) assess the area at risk, 2)
determine the degree of flow restoration soon after reperfusion therapy, and 3) estimate the degree of myocardial salvage after reflow would, indeed, be clinically valuable.

Myocardial thallium-201 (TI-201) imaging has been used to evaluate the efficacy of thrombolytic therapy in acute myocardial infarction.\textsuperscript{3-6} In patients demonstrating successful reperfusion, improved TI-201 uptake compared with pretreatment images are observed.\textsuperscript{3-5} Patients with a persistently occluded infarct-related vessel show little change in defect size.\textsuperscript{4,5} There are some significant limitations to the use of rest TI-201 scintigraphy for evaluating myocardial salvage after coronary reperfusion.\textsuperscript{7} First, because of prominent redistribution, it is necessary to obtain pretreatment images that may delay for up to 20–30 minutes the institution of thrombolytic therapy. Second, the physical characteristics of TI-201 are not optimum for imaging with a gamma scintillation camera. Third, not all persistent defects observed on serial resting TI-201 images reflect irreversible cellular injury. A significant percentage of persistent defects as observed visually show improved uptake after revascularization and actually represent ischemia and not necrosis.\textsuperscript{8,9} Fourth, if TI-201 is administered early during reperfusion, the degree of salvage could be overestimated because of "excess" TI-201 uptake during the phase of hyperemia.\textsuperscript{10-12} Finally, quantitation of TI-201 uptake and washout are required to optimally assess the degree of regional myocardial ischemia and infarction.\textsuperscript{13-15}

The 99mTc-labeled isonitrites are a new class of myocardial imaging agents that, because of superior physical characteristics, may be more optimal for myocardial perfusion imaging than TI-201. One of these agents, 99mTc-methoxyisobutyl isonitrile (99mTc-MIBI) is presently under investigation and appears promising for assessment of myocardial ischemia and infarction.\textsuperscript{16-20} 99mTc-MIBI remains relatively fixed in myocardial cells after initial extraction with minimal delayed redistribution.\textsuperscript{21,22} Thus, the radionuclide has the potential to be superior to TI-201 for evaluation of initial risk area and degree of improvement in flow and extent of salvage after reperfusion.\textsuperscript{23} One can administer the first dose of 99mTc-MIBI just before thrombolytic therapy but can postpone imaging for several hours in order not to delay treatment. The extent of hypoperfusion that exists before institution of reperfusion can be imaged after completion of the thrombolytic protocol.\textsuperscript{24,25} A second dose of 99mTc-MIBI can be injected after thrombolytic therapy, which should delineate the degree of flow improvement and extent of myocardial salvage.\textsuperscript{24,25}

The hypothesis tested in this experimental study is that 99mTc-MIBI administered during sustained coronary occlusion in open-chest dogs should accurately define the area at risk, whereas when administered after coronary reperfusion, the uptake pattern of the radionuclide should reflect degree of irreversible myocardial injury. In these experiments, we sought to determine whether or not 99mTc-MIBI uptake after reflow reflects merely the degree of flow restoration or extent of viable myocardium.

**Methods**

**Surgical Preparation**

Experiments were performed in 17 fasting adult mongrel dogs (mean weight, 27±5 kg) anesthetized with intravenous sodium pentobarbital (30 mg/kg). Animals were intubated and mechanically ventilated on a respirator (Harvard Apparatus, South Natick, Mass.) supplemented with 95% oxygen. A limb lead of the electrocardiogram was monitored continually. A femoral vein was isolated and cannulated with an 8F polyethylene catheter for the administration of fluids, medication, and 99mTc-MIBI. Both femoral arteries were isolated and cannulated with 8F polyethylene catheters for simultaneous withdrawal of microsphere reference samples; serial determination of arterial pH, partial pressure of carbon dioxide (PCO\textsubscript{2}), partial pressure of oxygen (PO\textsubscript{2}), and 99mTc-MIBI levels; and continuous arterial pressure monitoring. A balloon flotation catheter was positioned in the pulmonary artery for the measurement of cardiac output and central temperature.

A thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending artery (LAD) was isolated after the first diagonal branch, and an ultrasonic flow probe (T201, Transonic Systems, Inc., Ithaca, N.Y.) and snare ligature were placed around the vessel. A flared polyethylene tube was placed in the left atrial appendage for pressure measurement and for the injection of radiolabeled microspheres. All experiments were performed with approval of the University of Virginia Animal Research Committee in compliance with the position of the American Heart Association on research animal use.

**Experimental Protocol**

After baseline steady-state measurements, all dogs underwent 3 hours of sustained LAD occlusion, followed by 3 hours of reperfusion. Reperfusion was accomplished by release of the coronary snare. Dogs were premedicated with lidocaine (1–2 mg/kg, i.v.) 15 minutes before occlusion and reperfusion. They were divided into two groups (see Figure 1). Group 1 dogs were injected with 36±5 mCi of 99mTc-MIBI intravenously after 150 minutes of LAD occlusion. Group 2 dogs, which also underwent 3 hours of LAD occlusion, received 33±3 mCi of 99mTc-MIBI at 90 minutes of reperfusion after resolution of the hyperemic phase of reperfusion as monitored by the flow probe. In both groups of dogs, 2.0 ml arterial blood samples were serially withdrawn after injection of 99mTc-MIBI for the determination of blood clearance of the radionuclide. Radiolabeled microspheres (2–10 million; mean diameter, 11 µm) suspended in 10% dextran and Tween-80 were administered over 15 seconds into the left atrium in both groups of dogs during occlusion and reperfusion for assessment of
regional myocardial blood flow by methods previously described. The first two group 1 dogs studied were not injected with radiolabeled microspheres during reperfusion, although reperfusion was verified in these dogs by the ultrasonic flow probe.

Hemodynamics were recorded every 5 minutes during the 30-minute baseline period and every 15 minutes during occlusion and reperfusion periods. Cardiac output, central body temperature, and arterial blood gases were measured at hourly intervals. The ventilator was adjusted and bicarbonate was administered to maintain the pH, PO₂, and PCO₂ within the physiological range.

Postmortem Analysis

After completion of the protocol, the hearts were rapidly excised and the LAD reoccluded for postmortem dual perfusion to define the anatomic area at risk and the infarct area. The left main coronary artery was isolated, and a flared polyethylene catheter was secured in the ostium with a ligature. A second flared polyethylene catheter was inserted and secured into the LAD distal to the occlusion. The hearts were suspended in a bath of normal saline at 38° C. The dual perfusion was performed by simultaneous infusion of a phosphate-buffered solution of monastral blue into the left main coronary artery and a phosphate-buffered solution of triphenyltetrazolium chloride (TTC) into the LAD distal to the occlusion, under physiological pressures, for 5 minutes. Both buffered solutions were isosmotic and had a neutral pH. The hearts were incubated for an additional 15 minutes.

The hearts then were divided from base to apex into four slices of equal thickness (1–1.5 cm thick), which were photographed for later analysis. Slices were covered with plastic wrap and mounted on cardboard for imaging under a gamma scintillation camera (Technicare, Cleveland, Ohio) using an all-purpose low-to-medium energy collimator with a 20% window centered around the 140-keV peak of ⁹⁹ᵐTc-MIBI. All images were recorded using a 256×256 matrix with collection of 1×10⁶ counts. The slices then were placed in contact with high sensitivity x-radiographic film (AR Xomat, Kodak, Rochester, N.Y.) for 18 hours at room temperature for macroautoradiography. An acetate sheet was placed on the surface of the myocardial slices previously in contact with the radiographic film, and the endocardial and epicardial borders were carefully traced.

Correlation of ⁹⁹ᵐTc-MIBI Autoradiographs With Anatomic Risk Area and Infarct Area

The ⁹⁹ᵐTc-MIBI macroautoradiographs and the superimposed overlay of the endocardial and epicardial borders were placed on a light box for digitization on a computer image processing system (Mipron System, Kontron Electronics, Eching, FRG). Images were displayed using standardized histogram range expansion of the image gray scale to 256 gray levels. The ⁹⁹ᵐTc-MIBI autoradiographic defect area was planimetered from the enhanced images independent of the anatomic analysis.

The anatomic area at risk and infarct area were planimetered from 8×10 color photographic enlargements of each slice, using the same image processing system. The mass of myocardium at risk or infarcted for each slice was calculated by multiplying the weight of each slice by the average percentage of surface area either at risk or infarcted, as derived from analysis of the top and bottom surfaces of each slice. A composite value for each heart was calculated by summing the values from each of the four slices. The area at risk and infarct area planimetered from the top surface of each slice that was in direct contact with the radiographic film were correlated with the ⁹⁹ᵐTc-MIBI defect area planimetered from the enhanced macroautoradiographs.

Determination of Regional Myocardial Blood Flow and ⁹⁹ᵐTc-MIBI Activity

Each myocardial slice was divided into eight transmural sections, which then were subdivided into epicardial, midwall, and endocardial segments, resulting in a total of 96 segments for each dog. Gamma well scintillation counting of the myocardial segments was performed at 48 hours for the measurement of ⁹⁹ᵐTc-MIBI activity. The samples were
counted again 1 week later for the determination of microsphere flow, using a Packard gamma autoscintillation counter (Packard Instrument Co., Inc., Meriden, Conn.). Separation of isotopes by energy windows (\(^{99m}\)Tc-MIBI, 130–170 keV; \(^{103}\)Ru, 450–550 keV; \(^{85}\)Nb, 680–840 keV) was performed according to the methods of Heymann et al.,

To facilitate comparisons between dogs, regional myocardial blood flow and \(^{99m}\)Tc-MIBI activities were expressed as a percentage of control nonischemic values derived from a circumscribed region (15 segments) in the center of the left circumflex perfusion zone. Myocardial \(^{99m}\)Tc-MIBI uptake, expressed as a percentage of nonischemic uptake, then was correlated with microsphere-determined myocardial blood flow expressed as a percentage of nonischemic flow.

Myocardial segments were divided arbitrarily into the following zones based on microsphere flow during occlusion: severely ischemic (≤30% nonischemic), moderately ischemic (>30% but ≤60% nonischemic), mildly ischemic (>60% but ≤90% nonischemic) and nonischemic (>90% but ≤120% nonischemic). Myocardial \(^{99m}\)Tc-MIBI uptake in these flow ranges was calculated for each dog and correlated with microsphere flow.

**Statistical Analysis**

All computations were performed on a VAX computer. All data are presented as the mean±one standard deviation. Normality of the distribution was verified with either the Wilk-Shapiro test or the Kolmogorov-Smirnov test, depending on the population size. Univariate analysis of groups was performed by a paired Student's t test, unpaired two-sample t test, Wilcoxon signed rank test, or Wilcoxon rank signed test (statistical package, RS/1 Bolt, Bannock, Newman, Cambridge, Mass.). Differences between groups were considered significant at \(p<0.05\) (two-tailed). Linear regression analysis was used to compare paired samples and the correlation coefficient(s) expressed. When distributions were not normal, a Spearman rank correlation was used and Spearman's \(\rho\) calculated.

**Results**

Among the 17 dogs, three developed sustained ventricular arrhythmias, could not be immediately resuscitated, and were excluded. Of the remaining 14 dogs, seven were injected with \(^{99m}\)Tc-MIBI during occlusion (group 1) and seven during reperfusion (group 2). As previously cited, the extent of myocardium at risk and extent of myocardium infarcted were assessed by postmortem dual perfusion of monastral blue and TTC. Because our intent was to create a model with substantial myocardial infarction, one dog in each group was excluded from subsequent analysis because the mass at the myocardium infarcted was less than 10% of the area at risk. Among the remaining 12 dogs, the area at risk was 35±4% and 33±9% of the left ventricular mass for group 1 and group 2 dogs, respectively (\(p=NS\)). Infarct size was 45±11% of the area at risk among group 1 dogs and 49±5% among group 2 dogs (\(p=NS\)). Infarct mass also was comparable between groups when expressed as a percentage of total left ventricular mass (16±5%, group 1; 16±4%, group 2, \(p=NS\)). These results demonstrate that the groups had comparable risk areas and infarct sizes.

** Hemodynamics**

Heart rate and mean systemic arterial pressure remained constant throughout the experimental period for both groups. LAD flow measured by the flow probe immediately distal to the point of occlusion was at baseline 29±7 ml/min and 28±11 ml/min, respectively, for group 1 and 2 dogs. In both groups, distal LAD flow fell to zero during the 3-hour occlusion. There was a hyperemic response on release of the occlusion, with peak hyperemic flows of 2.5 times baseline, which slowly resolved. Among group 2 dogs, LAD flow at 90 minutes of reperfusion during \(^{99m}\)Tc-MIBI injection was 29±13 ml/min, not significantly different from flow at baseline. In all dogs, reperfusion was verified by the Doppler flow probe.

**Laboratory Evaluation**

The hematocrit, arterial pH, \(\text{PO}_2\) and \(\text{PCO}_2\) remained stable throughout the experiment. The thermodilution cardiac output was 3.5±0.9 l/min during baseline, fell to 3.0±0.9 during occlusion, and decreased further to 2.6±0.6 during reperfusion. Average central body temperature remained above 35° C throughout the experiment.

Arterial blood levels of \(^{99m}\)Tc-MIBI after the intravenous administration of the radionuclide were available in 10 of the 12 dogs included in this analysis. The arterial blood activity of \(^{99m}\)Tc-MIBI peaked within 30 seconds of injection and fell to 4% of peak activity within 5 minutes and to 2% of peak activity 10 minutes after intravenous administration. Among group 1 dogs, reperfusion occurred 30 minutes after the injection of \(^{99m}\)Tc-MIBI when blood activity was less than 1% of peak activity.

**Correlation of \(^{99m}\)Tc-MIBI and Regional Myocardial Blood Flow**

Group 1. Among dogs injected during LAD occlusion, the myocardial uptake of \(^{99m}\)Tc-MIBI in all endocardial segments (\(n=160\)) expressed as a percent of nonischemic uptake correlated well (\(r=0.91\)) with microsphere flow (percent nonischemic) at the time of injection (Figure 2A). The \(^{99m}\)Tc-MIBI activity from one dog was unavailable because of a malfunction of the gamma well counter. Close examination of the scatterplot (Figure 2A) of MIBI uptake versus occlusion flow demonstrates that the distribution falls along the line of identity, although \(^{99m}\)Tc-MIBI activity slightly exceeds flow in those endocardial segments with flow reduced to 25% or less of nonischemic flow. Transmural myocardial \(^{99m}\)Tc-MIBI activity (percent nonischemic) also closely cor-
related ($r=0.91$) with microsphere flow expressed as a percent of nonischemic flow (Figure 2B). The regression line defined by the relation of transmural $^{99m}$Tc-MIBI activity and flow approximated the line of identity (Figure 2B).

As illustrated in Figure 3, all segments were separated into severely ischemic, moderately ischemic, mildly ischemic, and nonischemic zones based on the selected occlusion microsphere flow ranges. The average myocardial $^{99m}$Tc-MIBI activity in each zone was calculated for each dog. In all zones, the regional myocardial $^{99m}$Tc-MIBI activity at the end of the experiment was not significantly different from microsphere flow at the time of injection during occlusion. In the severely ischemic zone, flow during occlusion was reduced to $8.6\pm2.2\%$ of nonischemic and increased to $67.4\pm4.1\%$ of nonischemic flow after reperfusion. Myocardial $^{99m}$Tc-MIBI activity in this zone remained depressed at $12.0\pm4.7\%$ of nonischemic 3.5 hours later, in spite of reperfusion. Myocardial $^{99m}$Tc-MIBI activity in the moderately and mildly ischemic zones was, respectively, $36.4\pm11.4\%$ and $75.8\pm11.5\%$ of nonischemic activity, which was comparable to the corresponding occlusion flows of $46.7\pm2.0\%$ and $78.5\pm0.8\%$ of nonischemic flow.

Figure 2. Myocardial $^{99m}$Tc-methoxyisobutyl isonitrile (MIBI) activity (% nonischemic) versus occlusion microsphere flow (% nonischemic) in group 1, which received MIBI during occlusion. Panel A: The relation between $^{99m}$Tc-MIBI uptake and occlusion flow at time of injection for all endocardial segments ($n=160$) among group 1 dogs. Panel B: The relation of transmural myocardial $^{99m}$Tc-MIBI activity and occlusion flow at time of injection. There was a close linear correlation of $^{99m}$Tc-MIBI myocardial activity and occlusion flow among both endocardial and transmural segments. Dotted line is line of identity.

Figure 3. Comparison of myocardial $^{99m}$Tc-methoxyisobutyl isonitrile (MIBI) activity (solid bars) with occlusion (hatched bars) and reperfusion (open bars) flows among segments from group 1 dogs segregated based on the occlusion flow ranges defined. All values for $^{99m}$Tc-MIBI activity and flow expressed as a percentage of control nonischemic values. Number of segments ($n$) analyzed for each flow range listed below each bar. Comparisons were performed on average values, computed for each dog for each occlusion flow range. *p<0.05 vs. $^{99m}$Tc-MIBI activity; −p<0.05 vs. occlusion microsphere flow.
Thus, these data demonstrate that when 99mTc-MIBI was administered during coronary occlusion, uptake correlated well with occlusion flow at the time of tracer injection, even when 3 hours of reperfusion ensued before killing the animals and counting the myocardial samples. This suggests that in this model of transmural myocardial infarction, there is no appreciable redistribution of 99mTc-MIBI as a result of reperfusion.

Group 2. When group 2 dogs were injected with 99mTc-MIBI after 90 minutes of reperfusion, the myocardial uptake of 99mTc-MIBI in endocardial segments (n=192) correlated poorly (r=0.66) with reperfusion flow (percent nonischemic) at the time of injection (Figure 4A). Transmural myocardial 99mTc-MIBI activity (percent nonischemic) also correlated poorly (r=0.56) with microsphere flow (percent nonischemic) at time of injection (Figure 4B). In each group 2 dog, endocardial segments could be identified that were completely nonviable (TTC negative). In Figure 4A, the endocardial segments within the risk area that were completely necrotic are identified by the filled circles. These segments, which represent the reperfused area of necrosis, demonstrated the lowest 99mTc-MIBI activity, in spite of reperfusion.

In the severely ischemic zone, 99mTc-MIBI activity after injection at 90 minutes of reperfusion was 25.1±4.8% of nonischemic activity, significantly less (p=0.002) than reperfusion flow, which was restored to 73.7±24.2% of nonischemic flow (Figure 5). Myocardial 99mTc-MIBI activity in the moderately and mildly ischemic zones was 53.9±12.1% and 83.6±5.6% of nonischemic, respectively, which also was significantly less than relative myocardial flow during reperfusion in these zones.

Although 99mTc-MIBI was injected during reperfusion, endocardial and transmural uptake of 99mTc-MIBI among group 2 dogs correlated better with flow measured during occlusion than during reperfusion (Figures 5, 6A, and 6B). Those segments among group 2 dogs with the lowest flow during occlusion had the lowest 99mTc-MIBI uptake during reperfusion (Figures 6A and 6B), in spite of restoration of flow. This suggests that the myocardial uptake of 99mTc-MIBI during reperfusion is dependent on the extent of irreversible injury and not just flow at the time of injection.

**Correlation of 99mTc-MIBI Autoradiographic Defect Area With Postmortem Anatomic Risk Area**

Group 1. The planimetered 99mTc-MIBI autoradiographic defect area was compared on a slice-by-slice basis with the anatomic risk area defined by planimetry of the postmortem dual perfusion maps (Figure 7). Among the 24 slices analyzed from group 1 dogs, the 99mTc-MIBI autoradiographic defect area (expressed as a percent of slice area) correlated well (r=0.94) with the anatomic risk area (percent of slice area) as reflected from monastral blue staining. As illustrated by Figure 7, the in vivo risk area quantitated by 99mTc-MIBI autoradiography was 29% smaller than the anatomic risk area (p=0.03). The ultimate infarct size determined by the TTC negative area correlated with the risk area defined by MIBI autoradiography (r=0.96) and the risk area defined by postmortem staining (r=0.95). On a slice-by-slice analysis, the infarct size was approximately 51% of the anatomic risk area and 65% of the risk area defined by MIBI autoradiography.
Figure 8 depicts examples of a postmortem dual perfusion pattern and a $^{99m}$Tc-MIBI autoradiograph from a representative group 1 dog in which $^{99m}$Tc-MIBI was injected during LAD occlusion. The autoradiograph is displayed with the superimposed epicardial and endocardial borders drawn. In this example, no significant $^{99m}$Tc-MIBI activity is observed on the autoradiograph corresponding to the anatomic risk area. Some dogs demonstrated a central defect with a faint rim of MIBI activity along the epicardial surface, presumably reflecting uptake via collateral flow during occlusion. To evaluate the presence of collateral flow, we separately analyzed flow in those transmural segments completely confined to the anatomic risk area. Among these segments, epicardial flow during coronary occlusion was $38\pm 27\%$ of nonischemic, which was significantly greater ($p \leq 0.0001$) than endocardial flow ($8\pm 16\%$ nonischemic).

Group 2. In these animals receiving $^{99m}$Tc-MIBI during reflow, the planimeted $^{99m}$Tc-MIBI autoradiographic defect area was compared on a slice-by-slice basis with the TTC-determined infarct area. Among the 24 slices analyzed from group 2 dogs, the $^{99m}$Tc-MIBI autoradiographic defect area correlated extremely well ($\rho = 0.98$) with the infarct area (Figure 9). The $^{99m}$Tc-MIBI autoradiographic defect area was not significantly different from the infarct area ($p = 0.50$). Figure 10 shows an example of the postmortem dual perfusion map and $^{99m}$Tc-MIBI autoradiograph from a representative group 2 dog in which $^{99m}$Tc-MIBI was injected during reperfusion.
**Discussion**

The findings of the present study suggest that the administration of 99mTc-MIBI during coronary artery occlusion delineates the area at risk. 99mTc-MIBI administered 90 minutes after reperfusion reflects the extent of myocardial viability rather than merely reflecting the degree of flow restoration. These data support the concept that serial 99mTc-MIBI imaging during acute myocardial infarction can delineate the area of myocardium at risk during occlusion and subsequently provide an assessment of the degree of myocardial salvage after reperfusion.

**99mTc-MIBI Uptake During Coronary Occlusion**

In the present study, uptake of 99mTc-MIBI in endocardial myocardial segments highly correlated (r=0.91) with microsphere flow at the time of injection. In endocardial segments in the severely ischemic zone with very low flow, 99mTc-MIBI activity, expressed as a percent of nonischemic activity, slightly exceeded normalized flow. This may be related to higher 99mTc-MIBI extraction in areas of low flow and is comparable to what has been reported for TI-201.27 Postmortem autoradiograms are comparable to those reported by Li et al,22 Okada et al,21 and Verani et al,23 who also showed an excellent correlation between 99mTc-MIBI uptake and microsphere-determined flow during experimental coronary artery occlusion in dogs.

In dogs receiving 99mTc-MIBI 2.5 hours into a 3-hour occlusion, we found no evidence of significant delayed redistribution of the radionuclide after 3 hours of reperfusion. 99mTc-MIBI uptake was comparable to microsphere flow at the time of administration during occlusion, even though animals were not killed until after 3 hours of reperfusion had ensued. It is important to note that in this model there was extensive infarction and that reperfusion was initiated 30 minutes after the injection of 99mTc-MIBI, when circulating blood levels were low.

**Assessment of Risk Area During Coronary Occlusion With 99mTc-MIBI**

In the present study, the hearts were rapidly excised at the end of the experiments for the postmortem dual perfusion of monastral blue and TTC to identify the anatomic area at risk and the infarct area. Postmortem dual perfusion is an established technique for the evaluation of the anatomic area at risk.29-31 The risk area defined by this type of postmortem staining, however, does not reflect collateral flow that may be present in vivo.

Gamma camera imaging after the intravenous injection of 99mTc-MIBI may permit the noninvasive assessment of an in vivo area at risk. To evaluate the potential of 99mTc-MIBI for the estimation of the area at risk, autoradiography was performed on myocardial slices from dogs injected with 99mTc-MIBI intravenously during coronary occlusion (group 1). This type of macroautoradiography of myocardial slices has been used with 99mTc-labeled albumin microspheres32 and nonbiodegradable plastic microspheres33 and provides a topical map of the myocardial distribution of the radiotracer that can be easily correlated with histological and histochemical staining performed on the same tissue. High-resolution macroautoradio-
graphs are obtainable with $^{99m}$Tc because the radiographic exposure is primarily the result of internal conversion and Auger electrons with low penetrating power.\textsuperscript{34,35} The low energy emissions producing the autoradiographs, however, necessitate close and uniform contact between the specimen and film emulsion.

In this study, the $^{99m}$Tc-MIBI autoradiographic defect area after the intravenous injection of $^{99m}$Tc-MIBI during coronary occlusion was correlated with the anatomic risk area. We found that the planimeted $^{99m}$Tc-MIBI autoradiographic defect area correlated closely ($r=0.94$) with the anatomic risk area quantitated from the monastral blue region. However, the $^{99m}$Tc-MIBI risk area determined by autoradiography was 29\% smaller than the anatomic risk area. DeBoer et al\textsuperscript{32} similarly demonstrated that the postmortem area at risk overestimates the in vivo risk area defined by auto-

FIGURE 8. Postmortem dual perfusion maps (left panels) and $^{99m}$Tc-methoxyisobutyl isonitrile autoradiographs (right panels) from representative group 1 dog. Shown (left) are three slices oriented with the right ventricle on the left and the anterior wall on the bottom. Left anterior descending artery risk area is stained brick red with triphenyltetrazolium chloride. Infarct area is the pale unstained region within the risk area. Defects seen on unenhanced autoradiographs with superimposed overlays (right) correlate closely with the anatomic risk area. Figure is reduced 15\%. 
radiography of technetium-labeled human albumin injected into the left atrium during coronary occlusion. The smaller defect size on $^{99m}$Tc-MIBI autoradiography compared with the anatomic risk area most likely reflects collateral flow present during coronary occlusion when $^{99m}$Tc-MIBI was injected, although it could potentially reflect scatter of $^{99m}$Tc emissions. In our model, flow in the epicardial region of the risk area was relatively preserved during coronary occlusion, suggesting that the smaller size of the $^{99m}$Tc-MIBI risk area was related to collateral flow.

Other techniques have been evaluated to assess the in vivo physiological risk area in both experimental myocardial infarction and in clinical studies. Presently, no widely accepted technique is considered to be highly accurate for the evaluation of in vivo risk area. In experimental models, the in vivo injection of dye into the aortic root, followed by rapid arrest of the heart with concentrated potassium chloride, has been used to define a physiological risk area. Contrast echocardiography has been proposed as a clinical method for the assessment of the physiological risk area. This technique currently is limited by the need for arterial catheterization and aortic root injection of the contrast agent. Macrogaggregated albumin also has been used as an in vivo marker of area at risk, but again, administration of this agent also requires left heart catheterization and intracoronary administration.

Our autoradiographic studies of $^{99m}$Tc-MIBI distribution during coronary occlusion indicate the potential for accurately assessing the risk area using tomographic imaging techniques and intravenous tracer administration. Initial experience with $^{99m}$Tc-MIBI reported by Verani et al showed a good correlation between defect size assessed by single photon emission computed tomography (SPECT) after coronary artery occlusion in dogs and the size of the occluded coronary bed as assessed at postmortem examination. Similarly, in our study, gamma camera images of the myocardial slices showed that defect size correlated well with macroautoradiographic risk area in dogs injected with $^{99m}$Tc-MIBI during coronary occlusion (see Figure 11). Gibbons et al recently demonstrated the feasibility of assessing the myocardial area at risk with tomographic $^{99m}$Tc-MIBI imaging in patients presenting with acute myocardial infarction.

$^{99m}$Tc-MIBI Uptake During Reperfusion

In dogs receiving $^{99m}$Tc-MIBI 90 minutes after reperfusion preceded by 3 hours of LAD occlusion, the uptake of the radionuclide was less than reperfusion flow at the time of injection. For example, flow in the severely ischemic zone was restored to an average of 73% of nonischemic flow after reperfusion, whereas $^{99m}$Tc-MIBI uptake was only 25% of nonischemic uptake. In myocardial segments in which necrosis was observed by TTC staining, there was a marked reduction in $^{99m}$Tc-MIBI uptake relative to flow. Interestingly, the myocardial uptake of $^{99m}$Tc-MIBI administered during reperfusion correlated better with flow during occlusion than during reperfusion among all the ischemic segments (Figure 5). One of the major determinants of the extent of necrosis is the degree of collateral flow that is reflected by the degree of flow reduction. Those segments with the lowest flows with 3 hours of occlusion had the lowest $^{99m}$Tc-MIBI uptake during reperfusion in spite of restoration of flow. This would suggest that the myocardial uptake of $^{99m}$Tc-MIBI during reperfusion is dependent on myocardial viability and not just reperfusion flow. Thus, despite significant flow restoration with reperfusion, $^{99m}$Tc-MIBI uptake remained depressed and was only slightly higher than the flow at the end of the occlusion period.

In these dogs, $^{99m}$Tc-MIBI was injected 90 minutes after reflow, which permitted resolution of hyperemia that occurs early as a result of suddenly restoring flow through a totally patent vessel. Animals were killed 90 minutes later, because this is the time period after tracer injection that might be required for transporting a patient to the imaging laboratory and allows for sufficient clearance of $^{99m}$Tc-MIBI from the liver and lungs at rest to achieve an optimal heart-to-background activity ratio.

We previously reported that myocardial uptake of $^{99m}$Tc-MIBI was not altered during severe ischemia produced by a chronic low flow state and during severe postischemic dysfunction resulting from a transient 15-minute LAD occlusion. In these prior studies, $^{99m}$Tc-MIBI uptake was not depressed relative to flow, although severe regional systolic dysfunction was documented in both groups of animals in the absence of necrosis. In the present study,
FIGURE 10. Postmortem dual perfusion maps (left panels) and $^{99m}$Tc–methoxyisobutyl i sonitrile (MIBI) autoradiographs (right panels) from representative group 2 dog. Shown (left) are three slices oriented with the right ventricular on the left and the anterior wall on the bottom. Risk area is stained brick red with triphenyltetrazolium chloride (TTC). Infarct area is the pale unstained region within the risk area. Defects seen on unenhanced autoradiographs with superimposed overlay (right) correlate closely with the area of infarction defined by TTC staining. No significant $^{99m}$Tc-MIBI activity is seen in the central necrotic region, whereas some activity is seen in the perinecrotic area. Figure is reduced 15%.

Assessment of Infarct Size During Reperfusion With $^{99m}$Tc-MIBI

In group 2 dogs in which $^{99m}$Tc-MIBI was administered 90 minutes after reperfusion, the autoradiographic defect area highly correlated ($r=0.98$) with...
Sinusas et al  

**99mTc-MIBI: A Marker of Myocardial Viability**  

**FIGURE 11.** Comparability of $^{99m}$Tc-methoxyisobutyl isonitrile gamma camera images (left panels) and macroautoradiographs (right panels) from a representative group 1 dog (top panels) and group 2 dog (bottom panels).

The TTC infarct area. No $^{99m}$Tc-MIBI activity was observed on autoradiographs or gamma camera images in zones corresponding to the TTC area of necrosis. Myocardial activity of $^{99m}$Tc-MIBI was very low in those TTC negative segments in the central risk area. $^{99m}$Tc-MIBI uptake could be observed, however, in the viable subepicardial regions in the risk area that stained positively with TTC. $^{99m}$Tc-MIBI was, therefore, taken up in viable areas and was virtually absent in the necrotic areas.

Thus, the results of the present study suggest that the presence of myocardial uptake of $^{99m}$Tc-MIBI may be a marker of viability and not just a flow tracer like radioactive microspheres.

Our data is consistent with the work of Verani and colleagues, who demonstrated that with reperfusion after 2 hours of occlusion, the residual perfusion defect was less than half the size of the original occlusion defect and was similar in magnitude to the anatomic infarct size. As in the present study, the increase in flow after reperfusion was significantly greater than the increased uptake of $^{99m}$Tc-MIBI. Thus, data from these present experiments, from our previous study, and from other investigators suggest that the uptake of $^{99m}$Tc-MIBI after coronary reperfusion preceded by variable periods of coronary occlusion reflects the degree of necrosis and the degree of myocardial salvage and extent of residual viable myocardium.

**Myocardial Cellular Uptake of $^{99m}$Tc-MIBI: Comparison to Ti-201**

Leppo and Meerdink investigated the myocardial transmicrovascular transport of $^{99m}$Tc-MIBI and Ti-201 in a blood-perfused isolated rabbit heart model. The average net myocardial extraction for $^{99m}$Tc-MIBI was significantly less than for Ti-201. In this preparation, the capillary permeability for Ti-201 was higher than $^{99m}$Tc-MIBI, but the parenchymal cell permeability and volume of distribution of the isonitrile compound was much greater than Ti-201. The net result of these differences in myocardial cellular transport is that little difference is observed in myocardial uptake of the two agents when imaged in vivo.

In another study by Meerdink and Leppo using the same isolated rabbit heart model, hypoxia and ouabain had minor effects on peak myocardial extraction of $^{99m}$Tc-MIBI and the permeability-
surface area product. However, myocardial retention as indirectly assessed by net tissue extraction was increased. Maublant et al.22 showed that in myocardial cells in culture, cyanide and iodoacetate, which profoundly affect myocardial metabolism by inhibiting the respiratory chain and glycolysis, respectively, did not affect 99mTc-MIBI uptake and efflux in the presence of impaired contractile function. TI-201 uptake was inhibited by the combination of cyanide and iodoacetate. In this cultured myocardial cell preparation, ouabain did not affect 99mTc-MIBI uptake. In contrast, TI-201 uptake was affected by ouabain inhibition of sodium-potassium ATPase. Thus, it appears that in these in vitro preparations, the uptake mechanism of 99mTc-MIBI is less dependent on active transport processes than TI-201.

The conclusion one can draw from these experiments and the large animal studies previously cited is that as long as the myocardial cell membrane is intact, it will maintain normal 99mTc-MIBI uptake. Only irreversible myocardial membrane injury appears to significantly affect intracellular sequestration of 99mTc-MIBI. Our data are consistent with this conclusion. 99mTc-MIBI uptake was severely diminished only in zones of myocardial necrosis as assessed by TTC staining.

Clinical Implications

The concepts underlying the present study and those previously reported in the literature have been applied in a preliminary manner to clinical 99mTc-MIBI imaging for the noninvasive assessment of thrombolytic therapy in acute myocardial infarction.24,25 Wackers et al.24 reported the results of a multicenter trial in which serial quantitative planar imaging with 99mTc-MIBI was performed in patients with acute myocardial infarction who had symptoms for 4 hours or less. An initial intravenous injection of 99mTc-MIBI was administered before thrombolysis, with imaging performed several hours later. The perfusion defect delineated the initial area at risk, comparable to what was exhibited in the animal models described previously.22,23 Postreperfusion imaging was undertaken after repeat injections of 99mTc-MIBI 18–48 hours or 6–14 days later. The defect zones identified on posttreatment images represented the area of infarction that was presumably not salvaged by treatment. In the study by Wackers et al.,24 there was no delay in instituting prompt therapy because pretreatment images were acquired several hours after administration of recombinant tissue-type plasminogen activator. Because of the lack of significant redistribution of 99mTc-MIBI, the pretreatment images accurately reflected area at risk. Patients with a patent infarct artery had a significant decrease in defect size on repeat images. In patients with a persistently occluded vessel, average defect was unchanged. The final defect size on the postthrombolytic therapy images also correlated well with the final left ventricular ejection fraction. Similar findings were reported by Gibbons et al.21 in a study using serial 99mTc-MIBI tomographic imaging before and 6–14 days after thrombolytic therapy. The late defect size assessed by quantitative analysis of SPECT 99mTc-MIBI images correlated inversely with the late left ventricular ejection fraction.

Conclusions

Because of the superior image quality possible when 99mTc-MIBI with SPECT techniques are used17–19 and the fact that global and regional function can be assessed simultaneously,19 the clinical application of 99mTc-MIBI imaging in acute infarction should be most useful. The capability of assessing risk area during acute coronary occlusion and degree of myocardial salvage after reperfusion offers a noninvasive means to evaluate the efficacy of therapeutic interventions such as thrombolytic therapy or acute coronary angioplasty aimed at reducing myocardial ischemic injury.

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