

99mTc-Sestamibi Uptake and Retention During Myocardial Ischemia and Reperfusion

George A. Beller, MD; David K. Glover, ME; Nathaniel C. Edwards, MD; Mirta Ruiz, MD; Juris P. Simanis, BS; and Denny D. Watson, PhD

Background. 99mTc-methoxyisobutyl isonitrile (Sestamibi) is a new perfusion agent that has shown promise for the noninvasive detection of myocardial salvage after coronary reperfusion in acute myocardial infarction. The objective of this study was to further validate that myocardial uptake and retention of Sestamibi after reperfusion in a canine myocardial infarction model are markers of tissue viability. The hypotheses tested were that if Sestamibi is given early after reperfusion and myocardial uptake is quantitated soon afterward, the degree of ultimate myocardial salvage will be overestimated, and that there will be continued loss of myocardial Sestamibi from ischemic tissue during 3 hours of reperfusion due to accelerated release of Sestamibi from cells already irreversibly injured during the phase of coronary occlusion, reperfusion injury to myocytes still viable early after reflow, or a combination of both mechanisms.

Methods and Results. In protocol 1, 8.0 mCi Sestamibi was injected intravenously in anesthetized dogs 2-5 minutes after reperfusion preceded by 3 hours of left anterior descending coronary artery (LAD) occlusion. Animals were killed either 5 minutes (n=7) or 3 hours (n=9) after Sestamibi administration. Mean endocardial Sestamibi activity was 74±3% of nonischemic activity in dogs killed early and 31±2% of nonischemic activity in dogs killed late after Sestamibi administration, indicating myocardial loss of Sestamibi during 3 hours of reflow. Regional flow (percent nonischemic) at the time of Sestamibi administration (2-5 minutes after reperfusion) was comparable in dogs killed early (144±23%) and dogs killed late (118±4%, p=NS). In protocol 2, Sestamibi was given intravenously at baseline under normal conditions followed by 3 hours of LAD occlusion and either 4 (n=6), 30 (n=9), or 180 minutes (n=10) of reperfusion. At postmortem, myocardial slices were imaged for quantification of defect magnitude and regional flow (radiolabeled microspheres), and tissue Sestamibi activities were determined by gamma well counting. Coronary sinus Sestamibi activity was serially measured. In these dogs, which were preloaded with Sestamibi at baseline, 3 hours of LAD occlusion followed by 3 hours of reperfusion resulted in a loss of Sestamibi in the endocardial zone of the ischemic region to 40±6% of nonischemic levels (p<0.0001). This loss corresponded to a sustained elevation of coronary sinus activity throughout the reflow period. The loss of myocardial Sestamibi was significantly greater than that observed in dogs killed 4 or 30 minutes after reflow. Defect magnitude also worsened over 3 hours of reperfusion as assessed by gamma camera imaging of slices of the excised hearts.

Conclusions. These experimental data suggest that Sestamibi uptake and retention are dependent on myocardial viability as well as regional flow. If Sestamibi is administered early after reperfusion and imaging is performed soon afterward, the degree of myocardial salvage could be significantly overestimated. (Circulation 1993;87:2033-2042)

KEY WORDS • myocardial infarction • microspheres • reperfusion

Sestamibi (99mTc-methoxyisobutyl isonitrile) is a new myocardial perfusion agent that has shown promise for the noninvasive detection of regional myocardial perfusion and viability.1-4 Like 30Ti, myocardial uptake of Sestamibi after intravenous injec-
The overall objective of the current investigation was to better define myocardial Sestamibi uptake and retention after reperfusion in dogs undergoing 3 hours of coronary occlusion and to determine if myocardial \(^{99m}\text{Tc}-\text{Sestamibi}\) retention is a marker of viability. The specific hypotheses tested in this study were that if Sestamibi is given early after reperfusion and myocardial uptake is quantitated soon afterward, the degree of ultimate myocardial salvage will be overestimated; and there will be continued loss of Sestamibi during 3 hours of reperfusion due to accelerated release of Sestamibi from cells irreversibly injured during the phase of coronary occlusion, reperfusion injury to myocytes still viable early after reflow, or both.

**Methods**

**Surgical Preparation**

Experiments were performed in 43 fasted adult mongrel dogs that were anesthetized with sodium pentobarbital (30 mg/kg), intubated, and ventilated on a respirator (Harvard Apparatus, South Natick, Mass.) with 4 cm of positive end-expiratory pressure. Arterial blood gases were monitored with levels maintained at physiological ranges. Lead II of the ECG was continuously monitored. The right femoral vein was cannulated with an 8F polyethylene catheter for administration of fluids, medications, and \(^{99m}\text{Tc}-\text{Sestamibi}\). Both femoral arteries were isolated and cannulated with 8F polyethylene catheters, and they were used for collection of arterial blood samples and microsphere reference blood withdrawal. A 7F catheter was placed in the right femoral artery for continuous monitoring of arterial blood pressure.

The basic open-chest canine model used in these experiments has been previously described. Briefly, a thoracotomy was performed at the level of the fifth intercostal space, and the heart was suspended in a pericardial cradle. A flare-tipped polyethylene tube was inserted into the left atrium via the left atrial appendage for continuous pressure measurement and injection of radiolabeled microspheres. For experiments in which coronary sinus sampling was performed, the left jugular vein was exposed, and a 7F coronary angiographic catheter (Cordis, Miami, Fla.) was advanced through the right atrium and into the coronary sinus until its tip rested in close proximity to the left anterior descending coronary artery (LAD). An approximate 1.5-cm segment of the LAD was dissected free of the epicardium, and an ultrasonic flow probe (T201, Transonic Systems, Inc., Ithaca, N.Y.) and a snare ligature were placed around the vessel.

All experiments were performed with the approval of the University of Virginia Animal Research Committee in compliance with the position of the American Heart Association on the use of research animals.

**Protocol 1: Sestamibi Uptake and Retention When Administered Soon After Reperfusion**

Experiments were performed in 18 dogs. After instrumentation of animals, 30 minutes of steady-state hemodynamic measurements were made. After this baseline period, the LAD was occluded for 3 hours. The occluder was then released, and reperfusion was documented by the LAD ultrasonic flow probe. At 2–5 minutes of reperfusion, 8.0 mCi \(^{99m}\text{Tc}-\text{Sestamibi}\) was injected intravenously, followed within 30–90 seconds by a left atrial injection of microspheres. Dogs were killed at either 5 minutes (group 1, \(n=8\)) or 3 hours (group 2, \(n=9\)) after Sestamibi injection. The experimental protocol was prematurely terminated in one group 2 dog because of rupture of the LAD at the site of the snare occluder, resulting in a total of 17 dogs completing protocol 1.

**Protocol 2: Myocardial Retention of Sestamibi After Coronary Occlusion and Reperfusion When Administered in the Resting State Before Occlusion**

The experimental design for this protocol is shown schematically in Figure 1. These experiments differed from those in protocol 1 in that Sestamibi was administered during the baseline state under normal conditions before coronary occlusion. Hemodynamic measurements of systemic arterial pressure, heart rate, left atrial pressure, and LAD flow were made serially at regular intervals throughout the protocol. After baseline steady-state hemodynamic measurements, dogs received 8.0 mCi \(^{99m}\text{Tc}-\text{Sestamibi}\). Fifteen minutes later, all dogs underwent 180 minutes of sustained LAD occlusion, followed by either 4 minutes (group I, \(n=6\)), 30 minutes (group II, \(n=9\)), or 180 minutes (group III, \(n=10\)) of reperfusion. Reperfusion was monitored by the immediate increase in flow documented by the LAD ultrasonic probe.

In all three groups of dogs, serial 2.0-mL aliquots of coronary sinus blood were withdrawn beginning during baseline and continuing throughout the occlusion and reperfusion periods. Samples were withdrawn every 30 minutes during occlusion; every 15 seconds during the first 2 minutes of reflow; at 4, 6, 8, and 10 minutes after reflow; and then every 15 minutes for the remainder of the reperfusion period. Arterial blood samples were simultaneously withdrawn at each time point to serve as a reference. Radiolabeled microspheres were injected into the left atrium at the time points indicated in Figure 1.

**Determination of Regional Myocardial Blood Flow With Radioactive Microspheres**

The technique for assessment of regional myocardial blood flow by the radioactive microsphere technique has been previously described. A dose of spheres (2–5 million; mean diameter, 11 \(\mu\)m) was suspended in 10% dextran and Tween 80. Normal saline was added to bring the total volume to 3 mL. Uniform mixing was accomplished initially by mechanical agitation (Vortex Genie mixer, Scientific Products) and followed by back-and-forth hand agitation between two syringes attached to a three-way stopcock. The syringe with microspheres then was administered over 15 seconds into the left atrium. For flow determination, paired arterial reference samples were obtained by continuous arterial withdrawal (Harvard Apparatus) over 130 seconds, beginning 10 seconds before the injection of each set of spheres.

**Postmortem Analysis**

After completion of each experiment in the two protocols, dogs were killed with a lethal dose of pentobarbital, and their hearts were rapidly excised. For the hearts excised in protocol 2, the LAD was reoccluded, and the hearts were perfused with Monastral blue dye.
and incubated in 1% triphenyl tetrazolium chloride (TTC) using a postmortem dual-perfusion technique to delineate the anatomic area at risk and infarct areas. The details of this technique have been reported previously.\textsuperscript{16}

All hearts were divided from apex to base into four slices of approximately equal thickness, as described previously. For dogs in protocol 2, the slices were photographed, placed on cardboard, and covered with plastic wrap (Saran Wrap\textsuperscript{®}, Dow). The endocardial and epicardial borders of each slice, as well as the risk and infarct areas, then were carefully traced onto acetate sheets. The heart slices were imaged with a gamma camera (Technicare 420, Cleveland, Ohio) for maximal count time. An all-purpose, low-to-medium energy collimator with a 20% window centered around the 140-keV peak of \textsuperscript{99m}Tc-Sestamibi was used. All images were recorded using a 128×128 matrix for maximal count time.

To measure \textsuperscript{99m}Tc activity and microsphere-determined flow in the myocardial tissue samples, each of the four myocardial slices was divided into eight transmural sections, which were further subdivided into epicardial, midwall, and endocardial segments, resulting in a total of 96 myocardial segments for each dog. The myocardial segments were counted in a gamma well scintillation counter (MINAXI 5550, Packard Instruments, Downer's Grove, Ill.) within 24 hours. Coronary sinus and arterial blood samples were also counted for \textsuperscript{99m}Tc activity using the gamma counter technique within 12 hours of collection. For the myocardial counting, window settings were \textsuperscript{99m}Tc, 130–170 keV; \textsuperscript{113m}Sn, 340–440 keV; \textsuperscript{106}Ru, 450–550 keV; \textsuperscript{95}Nb, 640–840 keV; and \textsuperscript{34}Sc, 842–1,300 keV. Tissue counts were corrected for background, decay, and isotope spillover, and regional myocardial blood flow was calculated using a computer software program (PDECAD, Packard Instruments, Downers Grove, Ill.). The transmural regional flow values for a specific sample were derived from the average of epicardial, midwall, and endocardial values for that sample. To facilitate comparisons of tracer activity with flow, the Sestamibi activities and microsphere flows were normalized to the average value of 15–18 samples taken from the nonischemic region supplied by the left circumflex coronary artery. Myocardial Sestamibi uptake, expressed as percent of nonischemic uptake, then was correlated with microsphere-determined flow expressed as percent of nonischemic flow.

**Image Quantification of Defect Magnitude**

Images of the four myocardial slices were recorded immediately after excision of the heart using the image parameters as described. Neither thresholding nor filtering was applied to the images. The intensity of the perfusion defect relative to the contralateral nonischemic wall was calculated by drawing transmural regions of interest in the central ischemic and nonischemic regions identical to the radial myocardial sections cut out for gamma well counting. The image perfusion defect ratio was computed by dividing the average pixel intensity in the ischemic region of interest by the average pixel intensity in the nonischemic region of interest.

**Statistical Analysis**

All data were analyzed by computation on a VAX computer. All data are presented as mean±1 SEM. Normality of the distribution was verified with either the Wilk-Shapiro test or the Kolmogorov-Smirnov test depending on the population size. For protocol 1 experiments, univariate analysis of groups was performed by a paired Student's t test, unpaired two-sample t tests, or Wilcoxon signed-rank test (statistical package, RS/1, Bolt, Beraneck, Newman, Cambridge, Mass.). Differences between groups were considered significant at a value of two-tailed p<0.05. Linear regression analysis was used to compare paired samples and the correlation coefficients expressed. When distributions were not normal, a Spearman rank correlation and a Spearman's rho were calculated. For protocol 2, within-group comparisons were made using a paired t test. Between-group comparisons were made with ANOVA, and if differences were found, they were analyzed with an unpaired t test. All protocol 2 statistical analyses were performed using SYSTAT software (SYSTAT, Inc., Evanston, Ill.).

**Results**

**Protocol 1: Uptake and Retention When Administered Soon After Reperfusion and Dogs Killed Early (Group I) or Late (Group II) After Reflow**

Data from one group I dog were excluded because of malfunction of the gamma well counter during analysis of
myocardial segments for Sestamibi activity and regional flow. This yielded a total of seven dogs in group I.

**Hemodynamics.** Heart rate remained stable for the duration of the protocol in groups I and II. In group I, arterial blood pressure remained constant throughout the experiment, whereas in group II, mean aortic pressure fell slightly during the 180 minutes of reflow. In group I dogs, LAD flow was 19±1 mL/min at baseline and fell to 0 mL/min with LAD occlusion. Peak LAD flow measured 1 minute after release of the occlusion was 60±12 mL/min (p=0.0001 versus baseline). Mean LAD flow at the time of 99mTc-Sestamibi administration at 2–5 minutes of reflow was 46±4 mL/min (p=0.001 versus baseline). In group II dogs, mean LAD flow was 25±1.5 mL under baseline conditions, fell to 0 mL/min during total LAD occlusion, and increased to 55±9 mL/min at 1 minute after reflow (p=0.001 versus baseline). Mean LAD flow at the time of 99mTc-Sestamibi injection at 2–5 minutes after reflow was 40±3 mL/min, comparable to flow in group I dogs at the time of 99mTc-Sestamibi injection.

**Relation between myocardial distribution of Sestamibi and regional myocardial blood flow.** Figures 2A and 2B summarize mean values for occlusion flow, reperfusion flow, and Sestamibi activity in the central ischemic zone in the seven group I dogs and the nine group 2 dogs, respectively. Values are shown for endocardial and transmural regions for each group. As summarized in Figure 2A, flow in the endocardial region during occlusion in group I dogs was 9±2% of nonischemic flow, significantly less than the transmural occlusion flow, which was 20±3% of nonischemic flow (p=0.0005). Reperfusion flow in the ischemic region at the time of Sestamibi injection (2–5 minutes after reflow) was 144±23% of nonischemic flow in the endocardial region, not significantly different from the corresponding mean transmural flow during reperfusion (166±14% of nonischemic flow). Mean endocardial Sestamibi activity in group I dogs was 74±2% of nonischemic activity, and transmural activity was 75±3% of nonischemic activity (p=NS).

Endocardial and transmural occlusion flows in group II dogs were 8±1% and 19±2% of nonischemic flows, respectively, values similar to those observed in group I dogs during LAD occlusion (Figure 2B). Similarly, endocardial and transmural flows during reperfusion at the time of Sestamibi injection were similar to values recorded in group I dogs (endocardial, 118±4%; transmural, 133±3% of nonischemic). At the end of the 3-hour reperfusion in these group II dogs, endocardial and transmural flows fell to 56±4% and 60±2% of nonischemic values, respectively. Mean endocardial Sestamibi activity in group II dogs killed at 180 minutes after reperfusion was 31±3% of nonischemic activity, and transmural Sestamibi activity was 43±5% of nonischemic (p=0.001). These values were significantly lower than those found in group I dogs killed at 5 minutes after Sestamibi administration, despite the fact that both groups had similar durations of occlusion and reflow before Sestamibi administration. The only differences between group I and group II dogs were the times after Sestamibi injection at which the animals were killed. These data reflect loss of myocardial 99mTc-Sestamibi in group II dogs during 180 minutes of reperfusion.

**Comparison of Sestamibi activity during reperfusion in group I and group II dogs.** Figure 3 depicts 99mTc-Sestamibi activities in group I dogs killed early and group II dogs killed late after reperfusion according to the regional microsphere-determined flow values during LAD occlusion. Endocardial activity is shown as percent of nonischemic activity in samples with flows at 0–30%, 31–60%, 61–90%, and 91–120% of nonischemic posterior wall flow. In these flow zones, no difference was seen between group I dogs and group II dogs with regard to occlusion flow or flow at the time of Sestamibi injection soon after reperfusion. As shown, in the samples in which endocardial flow was reduced to 0–30% of nonischemic flow during occlusion, mean
Sestamibi activity after reperfusion in group I dogs was 74±3% of nonischemic compared with 31±2% of nonischemic (p=0.001) in group II dogs. Similarly, in the regions where the occlusion flow was reduced to 31–60% of nonischemic flow, endocardial Sestamibi activity was 94±2% of nonischemic in group I compared with only 66±4% of nonischemic for group II dogs (p=0.001) after reflow. Nonischemic anterior wall Sestamibi activity in group I dogs killed early after reperfusion was slightly but significantly greater (115±1%) than anterior wall nonischemic Sestamibi activity in group II (100±1% of nonischemic; p=0.004) dogs killed late after reflow. This most likely represents some Sestamibi washout from normal myocardium between 7–10 and 180 minutes after reperfusion.

To summarize, the data in Figure 3 demonstrate significantly lower Sestamibi activity relative to normal activity in group II dogs killed 3 hours compared with group I dogs killed 7–10 minutes after reperfusion. Because both groups of animals underwent the same duration of occlusion and the same timing of Sestamibi administration during early reflow, this difference in final Sestamibi activity appears to be secondary to loss of myocardial 99mTc-Sestamibi during the 3 hours of reperfusion. That is, there was a loss of cellular retention of the tracer in the reperfused ischemic region after initial uptake. Because higher Sestamibi levels in group I dogs killed early after reperfusion could be due to increased tissue uptake because of hyperemic flow in which Sestamibi was not extracted intracellularly but remained predominantly in the interstitial space during the time between tracer injection and termination of the experiment several minutes later, a second experimental protocol was carried out. In protocol 2, Sestamibi was loaded in the myocardium under normal conditions before LAD occlusion and subsequent reflow. The rationale for these experiments was to assess 99mTc-Sestamibi retention by cells rendered ischemic and reperfused that was independent of tracer administration under hyperemic flow conditions.

**Protocol 2: Myocardial Retention of Sestamibi After Coronary Occlusion and Reperfusion When Administered in the Resting State Before Occlusion**

**Hemodynamics.** The values for the hemodynamic variables of heart rate, systemic arterial and left atrial pressures, and LAD flow during the baseline, occlusion, and reperfusion periods are shown in Table 1. Group I dogs undergoing 3 hours of occlusion and 4 minutes of reperfusion, group II dogs undergoing 3 hours of occlusion and 30 minutes of reperfusion, and group III dogs undergoing 3 hours of occlusion and 3 hours of reflow had no significant change in heart rate after occlusion. As is typical for 3-hour occlusion experiments, there was a tendency for arterial blood pressure to fall and left atrial pressure to rise over the occlusion time period in all groups. The ultrasonically measured LAD flow fell to 0 mL/min during occlusion. As shown in Table 1, reperfusion was documented in all three groups with the ultrasonic flow probe. Group I dogs killed 4 minutes after reperfusion demonstrated higher flow values during reperfusion than the other two groups. Because these animals were killed at 4 minutes after reperfusion, flows remained in the hyperemic range.

**Regional myocardial blood flow values.** Regional blood flow values at baseline (when Sestamibi was injected), during occlusion, and during reperfusion just before termination of the experiment are summarized in Table 2. Endocardial and transmural flows are depicted for the nonischemic LAD region proximal to the occlusion, in the severely ischemic region where flow during occlusion was less than 30% of nonischemic flow, and in the mild-to-moderate ischemic region where flow was reduced during occlusion to 31–80% of nonischemic flow. Endocardial and transmural flows at baseline were comparable in all three regions of the heart. During coronary occlusion, flow was reduced to approximately 50% of nonischemic flow in the mild to moderate ischemic region and to 10% or less of nonischemic flow in the most severely ischemic region. After reperfusion, endocardial and transmural flow from severely ischemic and mild to moderate ischemic regions were hyperemic in group I dogs killed 4 minutes after reperfusion.

**Coronary sinus sampling.** The graph in Figure 4 displays the coronary sinus Sestamibi values, expressed as percentage of its corresponding arterial sample during the time of the experimental protocol. Each value on the graph represents an average from all dogs from all three groups at that time point. As shown, a minimal amount of Sestamibi appeared in the coronary sinus blood throughout the occlusion period. After reflow, there was an instantaneous transient increase in Sestamibi activity, which was followed by a sustained elevation of activity during the 3 hours of reperfusion. The coronary sinus Sestamibi activity after 180 minutes of reflow remained substantially elevated compared with arterial Sestamibi activity. These data are consistent with a continuous net efflux of Sestamibi from the myocardium into the coronary venous effluent during reperfusion.

**Sestamibi retention after 3 hours of coronary occlusion and varying periods of reperfusion.** The mean normalized endocardial and transmural Sestamibi myocardial tissue activities in normal and ischemic regions in groups I–III
TABLE 2. Regional Myocardial Blood Flows (mL/min/g) at Baseline, During Occlusion, and After Reperfusion in Dogs Receiving Sestamibi at Baseline Followed by 3 Hours of Coronary Artery Occlusion and Either 4 Minutes (Group I), 30 Minutes (Group II), or 3 hours (Group III) of Reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline flows</th>
<th>Occlusion flows</th>
<th>Reperfusion flows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endocardial</td>
<td>Transmural</td>
<td>Endocardial</td>
</tr>
<tr>
<td>Normal LAD region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.02±0.06</td>
<td>1.07±0.04</td>
<td>1.11±0.05</td>
</tr>
<tr>
<td>II</td>
<td>1.01±0.02</td>
<td>0.99±0.01</td>
<td>1.00±0.02</td>
</tr>
<tr>
<td>III</td>
<td>0.96±0.02</td>
<td>1.00±0.02</td>
<td>1.03±0.02</td>
</tr>
<tr>
<td>Mild-to-moderate ischemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.90±0.08</td>
<td>1.06±0.05</td>
<td>0.53±0.02†</td>
</tr>
<tr>
<td>II</td>
<td>0.91±0.03</td>
<td>0.96±0.02</td>
<td>0.57±0.02†</td>
</tr>
<tr>
<td>III</td>
<td>0.90±0.03</td>
<td>0.99±0.03</td>
<td>0.54±0.03†</td>
</tr>
<tr>
<td>Severe ischemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.87±0.09</td>
<td>1.04±0.06</td>
<td>0.08±0.03†</td>
</tr>
<tr>
<td>II</td>
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<td>0.93±0.04</td>
<td>0.07±0.01†</td>
</tr>
<tr>
<td>III</td>
<td>0.83±0.04</td>
<td>0.98±0.04</td>
<td>0.08±0.02†</td>
</tr>
</tbody>
</table>

LAD, left anterior descending.

*p<0.05, †p<0.005 vs. baseline flows.

are summarized in Figures 5–7. In these figures, the normal region was composed of myocardial specimens in the distribution of the LAD in which endocardial flow during occlusion remained above 0.8 mL·min⁻¹·g⁻¹. The mild-to-moderate ischemic region was defined as those with sample flows between 0.3 and 0.8 mL·min⁻¹·g⁻¹. The severely ischemic region was defined as those samples with flows of less than 0.3 mL·min⁻¹·g⁻¹ during the occlusion phase of the experiments.

In all three groups in this protocol, Sestamibi was preloaded by intravenous injection under baseline conditions before occlusion of the LAD. Note that in group I and group II dogs undergoing 3 hours of occlusion and 4 minutes and 30 minutes of reperfusion, respectively, after ⁹⁹mTc-Sestamibi loading (Figures 5 and 6), activity of the radionuclide was significantly reduced in ischemic zones compared with nonischemic activity. However, the loss of myocardial ⁹⁹mTc-Sestamibi was significantly greater at 3 hours of reperfusion (group III) compared with 4 minutes (group I) and 30 minutes (group II) after reflow. In the 10 group III dogs preloaded with ⁹⁹mTc-Sestamibi at baseline, 3 hours of occlusion followed by 3 hours of reflow resulted in a loss of ⁹⁹mTc-Sestamibi in the endocardial zone of the severely ischemic region to 40±6% of nonischemic and in the transmural region as 51±5% of nonischemic (Figure 7). By ANOVA, there was a highly significant difference among groups I–III (p=0.003) in endocardial Sestamibi activity after reperfusion in the severely ischemic region. By unpaired t testing, there was no difference in activity between the 4- and 30-minute reperfusion groups but significant differences between 4-minute and 3-hour reperfusion groups (p=0.002) and between 30-minute and 3-hour reperfusion groups (p=0.012). Similar significant differences by ANOVA were seen among the three groups.

![Figure 4. Bar graph of coronary sinus ⁹⁹mTc-Sestamibi values, expressed as percentage of corresponding arterial sample value over time post-⁹⁹mTc-Sestamibi administration in dogs undergoing 3 hours of left anterior descending coronary artery (LAD) occlusion and 3 hours of reperfusion. ⁹⁹mTc-Sestamibi was administered at baseline, before LAD occlusion.](http://circ.ahajournals.org/lookup/suppl/doi:10.1161/01.CIR.87.6.2038/-/DC1/fig4.png)
with regard to transmural Sestamibi activity ($p=0.002$). In the zone defined as showing a mild-to-moderate degree of ischemia, ANOVA showed no significant difference in either endocardial ($p=0.066$) or transmural ($p=0.062$) Sestamibi activities among the three groups of dogs, but the trend was for lower levels in the 3-hour compared with 4- and 30-minute reperfusion groups. Thus, despite the same duration of total LAD occlusion in all three groups of dogs, a significant loss of $^{99m}$Tc-Sestamibi from the ischemic zone occurred over 180 minutes of reflow. This loss of tissue $^{99m}$Tc-Sestamibi corresponded to significant release of $^{99m}$Tc-Sestamibi into the coronary sinus blood.

**FIGURE 5.** Bar graph of mean $^{99m}$Tc-Sestamibi values (percentage nonischemic [NI]) in group I dogs undergoing 3 hours of left anterior descending coronary artery (LAD) occlusion (OCC) and 4 minutes of reperfusion (RP) in normal samples (endocardial flow during OCC >0.8 mL/min/g), mild-to-moderate ischemic samples (endocardial flow during OCC between 0.3 and 0.8 mL/min/g), and in severely ischemic samples (endocardial flow during OCC <0.3 mL/min/g). $^{99m}$Tc-Sestamibi was administered at baseline conditions before LAD occlusion.

**FIGURE 6.** Bar graph of mean $^{99m}$Tc-Sestamibi values (percentage nonischemic [NI]) in group II dogs undergoing 3 hours of left anterior descending coronary artery (LAD) occlusion (OCC) and 30 minutes of reperfusion (RP). $^{99m}$Tc-Sestamibi was administered at baseline conditions before LAD occlusion. Mild-to-moderate ischemia, endocardial flow during OCC between 0.3 and 0.8 mL/min/g; severe ischemia, flow during OCC <0.3 mL/min/g.

**FIGURE 7.** Bar graph of mean $^{99m}$Tc-Sestamibi values (percentage nonischemic [NI]) in group III dogs undergoing 3 hours of LAD occlusion (OCC) and 3 hours of reperfusion (RP). $^{99m}$Tc-Sestamibi was administered at baseline conditions before LAD occlusion. Mild-to-moderate ischemia, endocardial flow during OCC between 0.3 and 0.8 mL/min/g; severe ischemia, flow during OCC <0.3 mL/min/g.

Gamma camera imaging of defect magnitude. Figure 8 summarizes the defect magnitudes determined from the image count ratio of the LAD to the left circumflex region. Note that the mean defect magnitude in group III dogs undergoing 180 minutes of reperfusion was significantly greater than the defect magnitude in the group I and group II dogs, which underwent 4 minutes and 30 minutes of reperfusion, respectively. Again, because all dogs in this protocol received $^{99m}$Tc-Sestamibi under normal conditions before coronary occlusion, the greater defect magnitude in group III animals reflected a loss of tissue $^{99m}$Tc-Sestamibi as a consequence of reperfusion.

**Infarct Size Determinations**

Infarct size by TTC staining in group II dogs undergoing 3 hours of LAD occlusion and 30 minutes of reperfusion was 15.0±3.4% of the left ventricle and 35.6±5.7% of the risk area. In group III dogs undergoing 3 hours of LAD occlusion and 3 hours of reflow, these values were 11.9±2.2% and 31.5±4.9%, respectively, and not significantly different from group II animals. In contrast, in group I dogs, which underwent
only 4 minutes of reperfusion after 3 hours of occlusion, infarct was significantly smaller at 3.5±3.0% of the left ventricle (p<0.05) and 8.6±7.6% of the risk area (p<0.03). However, it should be pointed out that infarct size measurement by the TTC technique is unreliable with very short periods of reflow because there is not enough time to wash out the lactic dehydrogenase enzyme from necrotic cells.

Discussion

Data from the present study provide additional evidence that 99mTc-Sestamibi uptake and retention are sensitive to myocardial viability as well as regional myocardial blood flow. Myocardial uptake of Sestamibi administered early after reperfusion was substantially greater than we observed previously when the radionuclide was administered 90 minutes after reflow in the setting of the same duration (3 hours) of preceding coronary occlusion.16 After initial uptake in reperfused myocardium, there was failure of myocardial retention of the radionuclide during the ensuing 3 hours of reflow. Similarly, there was substantial loss of myocardial Sestamibi activity during 3 hours of reflow when the radionuclide was administered under baseline conditions before the subsequent 3 hours of occlusion followed by reperfusion (see Figures 5–7). This loss of myocardial 99mTc-Sestamibi consequent to reperfusion was associated with a sustained elevation of coronary sinus 99mTc-Sestamibi activity during the reflow period. Corresponding to this loss of myocardial 99mTc-Sestamibi activity determined by in vitro gamma well scintillation counting was a worsening of defect magnitude over 3 hours of reperfusion as assessed by gamma camera imaging of transverse myocardial slices obtained from postmortem sectioning of the left ventricle. These experimental data suggest that Sestamibi administered very soon after reperfusion would yield myocardial uptake patterns overestimating the degree of myocardial salvage and underestimating infarct size.

The mechanism for a continued myocardial loss of Sestamibi activity into the coronary venous blood during reperfusion resulting in worsening of the defect magnitude is uncertain. Most likely, 99mTc-Sestamibi release is accelerated from cells that were irreversibly injured during the period of ischemia. In addition or alternatively, Sestamibi may be released from cells damaged by reperfusion injury.21–27 For this explanation to be credible, it must be assumed that early after reperfusion, cells sequestering Sestamibi, or retaining the radionuclide after preloading, were viable. Finally, during reperfusion, some Sestamibi could become trapped in compartments formed by microvascular disruption, hemorrhage, or edema that have poor access to circulating blood. Our experiments do not provide definitive evidence favoring any one of these proposed mechanisms. The fact that infarct size by TTC staining was comparable in dogs undergoing 30 minutes and dogs undergoing 3 hours of reperfusion suggests the higher Sestamibi values in the former were due to myocardial retention in zones that were irreversibly injured early after reflow.

Sestamibi Uptake and Retention as Indication of Viability

Prior studies have yielded convincing evidence that Sestamibi is a suitable agent for evaluation of myocardial perfusion and cellular viability.7–20 Piwnica-Worms et al7 investigated the fundamental myocellular uptake mechanism of Sestamibi and found that its transport involves passive distribution across plasma and mitochondrial membranes, and at equilibrium it is seques-
tered largely within mitochondria by the large negative transmembrane potentials. When plasma membrane potentials or mitochondrial membrane potentials are depolarized, there is inhibition of net uptake and failure of retention of Sestamibi. Mitochondrial membrane depolarization occurs with irreversible myocyte injury.

Metabolic derangements simulating ischemia or hypoxia can result in diminished Sestamibi uptake independent of flow. Sestamibi depletion has been observed in heart cells with carbonyl cyanide m-chlorophenylhydrazine (CCCP) and 2,4-dinitrophenol, both of which depolarize mitochondrial membrane potentials.9 Other agents such as rotenone, nigericin, and dimethylsulfoxide have no significant effect on Sestamibi retention because these latter metabolic inhibitors have no detrimental effects on the negative potential across the inner mitochondrial membrane.9 Similar findings were reported by Beanlands et al17 in the isolated rat heart preparation. Under constant flow conditions, sodium cyanide, a cytochrome c oxidase inhibitor, and Triton X-100, a sarcolemmal membrane detergent, reduced peak accumulation of 99mTc-Sestamibi and enhanced clearance kinetics. Thus, adverse metabolic conditions induced by chemical agents will result in inhibition of cellular Sestamibi uptake and failure of Sestamibi retention.

Several studies in intact animals have shown that the uptake of Sestamibi is proportional to regional myocardial blood flow and tissue viability.5,6,13–16,28 In low-flow regions that remain viable, myocardial uptake of Sestamibi is higher than regional flow, most likely secondary to the known increased extraction of diffusible indicators at low flows. This observation has also been made with 201TI administration under similar ischemic conditions.29 As expected, myocardial extraction of Sestamibi is reduced at high flows.30 Extraction of 99mTc-Sestamibi is unaltered by hypoxia or ouabain administration as evaluated in an isolated rabbit heart model.7 After initial myocardial uptake, the subsequent clearance of 99mTc-Sestamibi is slow.5,10,14 This results in minimal "redistribution" over time under conditions of transient ischemia or a sustained low-flow state.

Further evidence that myocardial Sestamibi uptake is dependent on cellular viability is that its uptake is not significantly affected by myocardial stunning. Sinusas et al28 reported that the administration of Sestamibi during reperfusion preceded by 15 minutes of coronary occlusion resulted in normal Sestamibi uptake in the region of "stunned" myocardium that exhibited severe posts ischemic systolic dysfunction. In this model of transient occlusion and reperfusion, Sestamibi uptake was comparable to 201TI uptake and proportional to regional flow. Myocardial Sestamibi uptake also was not adversely affected in a canine model of "short-term" hibernation.28 Sinusas et al28 showed preserved Sestamibi uptake in anesthetized dogs with a partial coronary stenosis that resulted in a reduction of flow to 60% of control flow. Despite severe systolic dysfunction, uptake was still proportional to the residual flow in the ischemic zone. Results from these experiments suggest that low-flow ischemia producing profound systolic dysfunction
does not affect Sestamibi uptake as long as myocardial cells remain viable. Despite significant ischemic or postischemic systolic dysfunction in these experimental models, the uptake of Sestamibi is preserved. The radionuclide is extracted intracellularly as long as mitochondrial and plasma cell membrane integrity is intact and nutrient blood flow persists.

**Sestamibi Administration After Reperfusion**

Several experimental and clinical studies have been undertaken to determine whether Sestamibi is a useful radionuclide agent for assessing the risk area during prolonged coronary occlusion and the amount of salvaged and viable myocardium after reperfusion. Verani et al. and Sinusas et al. found that the perfusion defect size when Sestamibi was injected during occlusion correlated well with the risk area determined by histochemical staining. Similarly, these authors found that when Sestamibi was administered after reperfusion, defect size correlated with pathological infarct size. Despite adequate flow restoration to the reperfused infarcted region after reperfusion in these experiments, irreversibly injured cells did not sequester Sestamibi. These data in intact animals further validate the concept that only viable myocytes are capable of extracting and retaining Sestamibi. Results of clinical studies in patients with acute myocardial infarction undergoing reperfusion therapy have validated these observations in the experimental laboratory. When Sestamibi is administered after thrombolytic therapy in patients with acute myocardial infarction in these studies, there was a close relation between final defect size and regional and global left ventricular function. The final defect size after reperfusion also correlated well with enzymatic infarct size.

Thus, the results of both experimental and clinical studies provide evidence that serial Sestamibi imaging provides an accurate assessment of myocardium at risk during coronary occlusion and ultimate infarct size and degree of myocardial salvage with reperfusion. The final defect size will presumably also reflect the degree of injury, if any, induced by reperfusion itself.

Finally, the experimental data reported in the present study yield further evidence that Sestamibi may be a valid viability agent when administered at an appropriate time point during the course of evolving ischemic injury in the setting of reperfusion. Our data confirm that uptake and retention of Sestamibi are not solely dependent on regional blood flow but also on myocardial cellular integrity. This conclusion is supported by the observation that when 99mTc-Sestamibi was loaded in the myocardium under normal baseline conditions, subsequent prolonged ischemia and reperfusion resulted in a progressive loss of Sestamibi from myocardial tissue. This cumulative loss yielded a defect comparable in severity to that seen when Sestamibi was first administered after the same duration of coronary occlusion (3 hours) and reflow (3 hours). These data support the hypothesis that necrotic cells can neither extract nor retain Sestamibi.

**Implications of the Present Study**

There are some relevant clinical implications to the observations made in the present experimental study. First, if Sestamibi is administered soon after reperfusion and imaging is performed within 30 minutes, the degree of myocardial salvage could be overestimated and infarct size will be underestimated. Data from this study show that when Sestamibi is administered immediately after reflow, defect magnitude is less and myocardial activity is substantially higher than when quantitated 3 hours later. Therefore, a rapid radionuclide imaging protocol aimed at assessing the efficacy of reperfusion soon after administration of a thrombolytic agent with Sestamibi may yield misinformation. These observations are similar to those made when 201TI is administered soon after reperfusion, resulting in overestimation of degree of salvage. The present experimental studies also demonstrate a considerable loss of Sestamibi over 3 hours of reflow, perhaps secondary to accelerated egress of Sestamibi from cells irreversibly injured during the phase of occlusion, some reperfusion injury, or both. A delay of at least 2–3 hours after restoring vessel patency before administering Sestamibi to noninvasively assess the amount of ultimate myocardial salvage would seem appropriate. If 99mTc-Sestamibi is administered soon after restoring blood flow, then a delay of at least 2–3 hours should be instituted before commencing imaging to accurately determine degree of myocardial salvage. Imaging at that time point would provide more accurate information pertaining to infarct size.

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