Relations of the Myocardial Imaging Agents $^{99m}$Tc-MIBI and $^{201}$Tl to Myocardial Blood Flow in a Canine Model of Myocardial Ischemic Insult

Robert C. Canby, MD, Sigmund Silber, MD, and Gerald M. Pohost, MD

Myocardial imaging with thallium 201 has proven to be an important clinical procedure to assess the severity of the myocardial ischemic insult. Uptake of $^{201}$Tl is related to perfusion and extraction by intact myocardium. Recently, a newer group of agents based on $^{99m}$Tc alkyl isonitriles has been developed and appears promising for myocardial imaging. Although the distribution of this new agent has been shown to be related to myocardial perfusion, its dependence on myocardial integrity has not been established. This study compared the distribution of $^{99m}$Tc-2-methoxy-isobutyl-isonitrile ($^{99m}$Tc-MIBI) with that of $^{201}$Tl in a clinically relevant canine model of ischemic insult. Fifteen adult dogs underwent 2 hours of occlusion by left anterior descending coronary artery ligation followed by reperfusion. In one group of dogs, $^{201}$Tl and $^{99m}$Tc-MIBI were administered 5 minutes before 35 minutes of reflow (group 1, n=5). In the other animals, the agents were given 5 minutes after onset of reflow, and dogs were killed after 10 (group 2, n=5) and 35 minutes of reperfusion (group 3, n=5). $^{99m}$Tc-MIBI activity was significantly correlated with $^{201}$Tl activity ($r=0.91$, 0.77, and 0.92, for groups 1, 2, and 3, respectively). Both $^{201}$Tl and $^{99m}$Tc-MIBI activities were correlated similarly with blood flow in all models. In groups 1 and 2, $^{201}$Tl and $^{99m}$Tc-MIBI activities correlated directly with microsphere-determined blood flow, whereas in group 3, they correlated inversely. The present study shows that in these models of myocardial ischemic insult, $^{99m}$Tc-MIBI distribution is closely related to that of $^{201}$Tl. (Circulation 1990;81:289–296)

Myocardial imaging with thallium 201 has been established as an important clinical tool for detection of and prognostication in coronary artery disease.1–6 However, a major limitation of $^{201}$Tl is that its lower photon energy is not optimal for myocardial imaging because of scatter and attenuation from overlying structures. Technetium 99m has a higher energy that is optimal for gamma camera imaging with substantially less attenuation.

Whereas the early $^{99m}$Tc-labeled complexes, such as DMPE, rendered suboptimal image quality, a new group of agents based on $^{99m}$Tc-labeled alkyl isonitriles has been developed that is promising for myocardial imaging.7–10 Whereas $^{99m}$Tc-hexakis-tertiary-butyl-isonitrile (TBI) and $^{99m}$Tc-hexakis-carboxymethoxy-isopropyl-isonitrile showed limitations relating to lung and liver uptake, the $^{99m}$Tc-hexakis-2-methoxy-2-isobutyl-isonitrile ($^{99m}$Tc-MIBI, RP30) showed rapid blood clearance and low lung and liver uptake.11–17 Therefore, $^{99m}$Tc-MIBI is the best of the clinically tested $^{99m}$Tc-isonitriles and has provided excellent myocardial image quality.12

Although experimental studies11,18 showed that the myocardial distribution of $^{99m}$Tc-MIBI is largely dependent on myocardial perfusion, there is no published study in which $^{201}$Tl and $^{99m}$Tc-MIBI were directly compared in the same animal using a model of coronary occlusion and reperfusion. The purpose of the present study is to compare the distribution of $^{99m}$Tc-MIBI with that of $^{201}$Tl and relate both to myocardial perfusion in clinically relevant canine models of myocardial ischemic insult, that is, 2 hours of coronary occlusion followed by reperfusion.

Methods

Fifteen adult mongrel dogs (mean weight, 22 kg) were anesthetized with sodium pentothal (20 mg/kg...
i.v.), intubated, and ventilated initially at 2 l/min with a mixture of 26% oxygen, 70% nitrous oxide, and 4% halothane with a volume ventilator (Harvard Apparatus, South Natick, Massachusetts) and a recirculating anesthetic system. The halothane concentration was decreased slowly to 1% in 3–5 minutes and was adjusted to maintain a moderately depressed eyelid reflex. The heart was exposed through a left thoracotomy and suspended in a pericardial cradle. A catheter (20 cm in length) was inserted into the left atrium for injecting microspheres. Another catheter (25 cm in length) was advanced from the right femoral artery to the descending aorta and attached to a Buchler polystaltic pump to obtain microsphere reference samples for later determination of regional myocardial blood flow and for obtaining blood samples for measurement of arterial pH, PCO₂, and PO₂. A third catheter (20 cm in length) was placed in the left carotid artery and advanced into the left ventricle for monitoring systolic and end-diastolic pressures. A large-bore cannula was inserted into the femoral vein for administration of saline, lidocaine, procainamide, and potassium chloride to maintain stable hemodynamic parameters, to prevent ventricular arrhythmias, and to maintain potassium levels within normal range. The left anterior descending coronary artery (LAD) and its major branches beginning just above the origin of the second diagonal branch were dissected free, and a loose suture was placed around each vessel. Left ventricular pressure and electrocardiographic lead II were monitored throughout the experiment and recorded on paper with a multichannel recorder (ES 1000 Electrostatic Recorder, Gould, Cleveland, Ohio). Throughout the course of study, arterial blood was sampled, and pH, PO₂, and PCO₂ were maintained within the following ranges: pH, 7.35–7.45; PCO₂, 30–40 mm Hg; and arterial PO₂ above 100 mm Hg.

Studies were performed according to three protocols with five dogs in each. In all protocols, a 2-hour occlusion interval was followed by reperfusion. Hepar (5,000 units) and procainamide (50 mg/kg) were administered intravenously before occlusion. After baseline measurements were obtained, steady-state hemodynamic measurements were obtained. To produce a large enough area of insult, the mid-LAD was ligated, followed by ligation of the marginal or diagonal branches. Additional procainamide was given 60 and 105 minutes after occlusion. Ten minutes before reflow, approximately 2 million ⁹⁵Nb-labeled microspheres (15 μm, Du Pont, North Billerica, Massachusetts) were injected into the left atrium. The microspheres were thoroughly suspended in 2 ml normal saline and 0.01% Tween-80 by agitating them for 5 minutes in a Vortex mixer before administration.

Figure 1 depicts the experimental protocols. Group 1 animals were given intravenous doses of ²⁰¹Tl (100 μCi) and ⁹⁹mTc-MIBI (15 mCi) 5 minutes before reflow and were killed 35 minutes after onset of reflow. This protocol compares the “redistribution” of the two agents. In groups 2 and 3 animals, ²⁰¹Tl and ⁹⁹mTc-MIBI were given intravenously 5 minutes after onset of reflow. The purpose of the group 2 and 3 protocols was to characterize the changes in myocardial content of ²⁰¹Tl and ⁹⁹mTc-MIBI with reflow. Group 2 animals were killed 10 minutes after the onset of reflow, whereas group 3 animals were killed 35 minutes after the onset of reflow. In all protocols, ⁸⁵Sr-labeled microspheres

![Figure 1. Schema of experimental protocols. See text for details.](http://circ.ahajournals.org/DownloadedFrom/circ.ahajournals.org)
201TI and 99mTc-MIBI were administered 5 minutes after onset of reflow. Thus, the distribution of these agents was measured at 5 minutes and 30 minutes after their administration. In this way, the impact of continued reflow on tracer distribution was evaluated providing comparative data on clearance from zones of myocardium that underwent reflow.

At the end of the experiment, the heart was removed and sectioned into four slices of approximately 1-cm thickness parallel to the left ventricular base. The left ventricular free wall was divided into the anterior zone (in the distribution of the ligated artery) and the posterior (or control) zone. The region bordered by the LAD and its second diagonal branch to the apex was chosen as the anterior zone. Control myocardium was obtained from the posterior wall. Four transmural anterior and four transmural posterior myocardial specimens were obtained and subdivided into endocardial, midwall, and epicardial segments. Thus, the total number of myocardial samples for each zone was 12. Each myocardial sample weighed between 1 and 2 g.

Regional Myocardial Blood Flow and Regional 201TI and 99mTc-MIBI Activities

Radioactivity counts from anterior and control zone samples were measured using a multichannel gamma well counter (model 1282, LKB). 99mTc-MIBI radioactivity was counted on the day after study, whereas the 201TI, strontium 85, and niobium 95 activities were counted 7 days later. The 201TI, 85Sr, 95Nb, and 99mTc-MIBI activities were computed with no correction for spillover. Regional myocardial blood flow was calculated for each myocardial sample using activities of reference blood samples obtained simultaneously with the administration of each isotope. 201TI, 99mTc-MIBI, and microsphere activities in each anterior zone sample were expressed as a percentage of the average of the posterior wall control samples for the respective endocardial, midwall, and epicardial segments.

Regional Myocardial Tissue Water Content

Both anterior and control zone samples were weighed and dried to constant weight at 105°C. Total tissue water content was calculated and expressed as milligrams of water per gram of dry weight. Water content for each anterior sample was expressed as a percentage of the average of the control samples for the respective endocardial, midwall, and epicardial layers.

Statistical Analysis

All results are expressed as mean±1 SD. The significance of a difference between means was assessed with Student’s t test. Significance was defined at the level of p<0.05. Correlations between 201TI and 99mTc-MIBI activities and regional myocardial blood flow were determined with linear regression analysis. The differences in regression lines were determined using an analysis of variance for differences between slopes and intercepts.

Results

Hemodynamic Data

The mean heart rate, left ventricular end-diastolic pressure, and mean arterial pressure did not differ significantly between study groups before occlusion, during occlusion, or after the onset of reperfusion. Mean heart rates for all groups were 110±13.9 initially, 104±14.3 after 2 hours of occlusion, and 121±40.9 beats/min after 10 minutes of reflow. Mean end-diastolic left ventricular pressures initially, after 2 hours of occlusion, and after 10 minutes of reperfusion were 8.5±3.6, 11.3±4.7, and 10.4±2.5 mm Hg, respectively. Mean arterial pressure before LAD ligation was 104±18 mm Hg. After 2 hours of occlusion, a small but nonsignificant decrease in mean arterial pressure was observed in all study groups with the mean value in all dogs of 97±20 mm Hg. Mean arterial pressure after 10 minutes of reperfusion was 99±13 mm Hg.

Relation of 99mTc-MIBI to 201TI Myocardial Distribution

In Figure 2, 99mTc-MIBI activity is plotted against 201TI activity in the anterior zone for each study group: group 1, where 99mTc-MIBI and 201TI were administered just before reflow; group 2, where 99mTc-MIBI and 201TI were administered 5 minutes after onset of reflow and followed by an additional 5 minutes of reflow; and group 3, where 99mTc-MIBI and 201TI were administered 5 minutes after reflow and followed by an additional 30 minutes of reflow. 99mTc-MIBI and 201TI activities are expressed as a percentage of the mean posterior wall value for each myocardial layer (endocardial, midwall, or epicardial). All posterior wall myocardial sample flows were 0.8 ml/g/min or higher. There was a significant linear relation between 99mTc-MIBI and 201TI activities for each of the three study groups with r=0.91 (p<0.0001) for group 1, r=0.77 (p<0.001) for group 2, and r=0.92 (p<0.0001) for group 3. The slopes and y intercepts of the lines for groups 1 and 3 are not significantly different from the line of identity. The slope and y intercept in group 2, however, are significantly different from the line of identity (p<0.05 for slope and y intercept).

Relation of the Myocardial Distribution of 99mTc-MIBI and 201TI to Regional Myocardial Blood Flow

In Figure 3, 99mTc-MIBI and 201TI activities are plotted against blood flow during occlusion in anterior samples for group 1. In Figures 4 and 5, 99mTc-MIBI and 201TI activities are plotted against myocardial blood flow during reperfusion for groups 2 and 3. The wide range of anterior zone blood flows is due to wide flow ranges in individual dogs rather than to wide variations between dogs. Although there are significant linear relations between 99mTc and 201TI activities and regional blood flow determined during occlusion in group 1, the distribution of the data points suggests a nonlinear pattern. Accordingly, a polynomial curve-fitting analysis was performed.
The solid line represents the lines of regression, and the dashed lines represent the line of identity. In all groups, $^{99m}$Tc-MIBI activity correlated significantly with $^{201}$Tl activity. $^{99m}$Tc-MIBI, $^{99m}$Tc-hexakis-2-methoxy-2-isobutyl-isonitrile.

In the upper panel of Figure 2, there are significant positive correlations between both $^{99m}$Tc-MIBI and $^{201}$Tl activity and microsphere activities ($r = 0.74$, $p < 0.005$, and $r = 0.75$, $p < 0.01$, respectively), in group 3 (Figure 5), there was a significant inverse correlation between $^{99m}$Tc-MIBI and $^{201}$Tl and microsphere activities ($r = -0.88$, $p < 0.0005$, and $r = -0.88$, $p < 0.0001$, respectively).

**Relation of $^{201}$Tl and $^{99m}$Tc-MIBI Distributions to Myocardial Water Content**

To further evaluate the possibility that edema is a factor responsible for differences in myocardial $^{99m}$Tc-MIBI and $^{201}$Tl content, myocardial water content was determined for the flow ranges and is depicted in Figure 3. There are increases in water content with each increment in reperfusion flow in both groups. The increase in water content parallels the increase in $^{99m}$Tc-MIBI and $^{201}$Tl activities in group 2 (Figure 6, upper panel) but is inversely related to tracer activity.
in group 3 (Figure 6, lower panel). Compared with samples taken at 10 minutes of reperfusion, samples taken at 35 minutes of reperfusion showed no significant difference in myocardial water for each flow range. For group 1, water content was inversely related to flow during occlusion and to $^{99m}$Tc-MIBI and $^{201}$Tl activities at the time of death.

Discussion

The present study compared the myocardial uptake of $^{99m}$Tc-MIBI, a new myocardial imaging agent, and $^{201}$Tl when administered before and after reperfusion in dogs subjected to a 2-hour occlusion of LAD. It shows that $^{99m}$Tc-MIBI and $^{201}$Tl distribute similarly in models of 2 hours of coronary occlusion with 10 minutes and with 35 minutes of reperfusion.

$^{201}$Tl behaves similarly to potassium in biologic systems. Several studies suggest that thallium uptake is related to viability in addition to regional perfusion. In a study by Goldhaber et al., in the fetal mouse heart organ culture preparation subjected to varying degrees of ischemic-like insult, $^{201}$Tl uptake at 2 hours showed a strong inverse relation with lactate dehydrogenase release 10 hours after onset of insult. Lactate dehydrogenase release is an excellent indicator of cell mortality. Uptake of thallium in this model is independent of flow because the reservoir bathing the hearts exposed them to a continuous thallium supply. Next, in a study by Khaw et al., the distribution of radiolabeled specific antibody to cardiac myosin showed a very close inverse correlation with $^{201}$Tl in a canine model of 28 hours of coronary artery occlusion 1 hour after $^{201}$Tl administration.

Because of its high first pass myocardial extraction efficiency, its initial uptake is directly related to regional myocardial blood flow distribution. Regions of relative myocardial underperfusion show up as defects on gamma camera images. Resolution or disappearance of such defects has been termed "redistribution." Redistribution is related to the combination of late uptake from noncardiac reservoirs via the blood and decreased myocardial clearance between the initial and delayed images. The uptake of $^{99m}$Tc-isonitiriles also is related to regional myocardial blood flow distribution; however, they are bound to intracellular proteins. In contrast to $^{201}$Tl, the exact mechanism of $^{99m}$Tc uptake in myocardium is not well understood. Early investigations suggest that myocardial uptake is predominantly passive and related to its lipophilic cationic nature. However, an active component recently has been suggested.
pared with $^{201}$Tl, the extraction fraction of $^{99m}$Tc-MIBI is lower (65%), and no significant redistribution occurs under clinical conditions.\textsuperscript{12}

**Group 1**

When given before reflow at the end of a 2-hour coronary occlusion interval, there is a close positive linear correlation between $^{99m}$Tc and $^{201}$Tl activities and microsphere-determined myocardial flow (Figure 3). As anticipated, extraction efficiency at low flows increases for $^{99m}$Tc-MIBI and $^{201}$Tl as suggested by the observation that the $y$ intercept is positive and is significantly different from zero. As evidenced by the third-degree polynomial curve-fitting analysis, $^{99m}$Tc-MIBI and $^{201}$Tl activities diverge from the line of identity, particularly at moderately decreased flows (Figure 3). These observations indicate that in zones in which moderate injury is anticipated (about 10–40% control flow), an excess of $^{99m}$Tc-MIBI and $^{201}$Tl occurs above the level predicted on the basis of blood flow measurements. In zones in which severe injury or infarction is anticipated (0–10% of control flow), the excess is less. $^{99m}$Tc-MIBI and $^{201}$Tl accumulation in the moderate injury zone suggests continued extraction of the agents by viable myocardium during the 40-minute interval after administration. The tendency for lesser accumulation in the severe injury zone may be related to the reduced ability of infarcted, nonviable myocardium to extract the two myocardial imaging tracers.

In view of the similar distribution of $^{99m}$Tc-MIBI and $^{201}$Tl approximately 30 minutes after onset of reflow and the similar relation of $^{201}$Tl and $^{99m}$Tc-MIBI to regional myocardial blood flow distribution at this time, the two agents appear to demonstrate the phenomenon of redistribution in a similar fashion. The reasons for $^{99m}$Tc-MIBI not appearing to redistribute in clinical studies perhaps can be related to the following. The blood levels of $^{99m}$Tc-MIBI after exercise administration are considerably lower relative to myocardial activity than the blood levels of $^{201}$Tl under similar circumstances. This could be due to the lack of substantial clearance from the large reservoir of the agent in the skeletal muscle after exercise. Redistribution of $^{99m}$Tc-MIBI was shown in a recent canine study after administration during a brief LAD occlusion when blood levels were increased.\textsuperscript{30} The observation that the curve better approximates the line of identity at very low flows also could be accounted for by the more rapid washout of the radiopharmaceuticals from the most severely injured cells. Finally, another potential factor is the clearance of $^{99m}$Tc-MIBI from the myocardium. If clearance is substantially slower than that of

---

**Figure 5.** Plots of relations of $^{99m}$Tc-MIBI activity (% control) in anterior myocardial samples (Panel a) and $^{201}$Tl activity (Panel b) to microsphere-determined blood flow (% control) in group 3. (For further explanation, see Figure 2.) $^{99m}$Tc-MIBI, $^{99m}$Tc-hexakis-2-methoxy-2-isobutyl-isonitrile.
Canby et al 99mTc-MIBI, Thallium 201, and Myocardial Blood Flow 295

TRACER COMPARED WITH WATER CONTENT
5 min after administration (group 2)

<table>
<thead>
<tr>
<th>Sr-85 microsphere determined blood flow</th>
<th>100-200</th>
<th>201-300</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-201 Tc-99m</td>
<td>100-200</td>
<td>201-300</td>
<td>300</td>
</tr>
<tr>
<td>Water Content</td>
<td>100-200</td>
<td>201-300</td>
<td>300</td>
</tr>
</tbody>
</table>

35 min after administration (group 3)

<table>
<thead>
<tr>
<th>Sr-85 microsphere determined blood flow</th>
<th>100-200</th>
<th>201-300</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-201 Tc-99m</td>
<td>100-200</td>
<td>201-300</td>
<td>300</td>
</tr>
<tr>
<td>Water Content</td>
<td>100-200</td>
<td>201-300</td>
<td>300</td>
</tr>
</tbody>
</table>

FIGURE 6. Upper panel: Tracer activity (% control) and myocardial water content (% control) in group 2 animals 5 minutes after tracer administration during reflow for anterior myocardial samples with 85Sr microsphere-determined blood flows of less than 100%, 100–200%, 201–300%, and greater than 300% of control (nonischemic) blood flow. Lower panel: Tracer activity (% control) and myocardial water content (% control) in group 3 animals 30 minutes after tracer administration during reflow for anterior myocardial samples with 85Sr microsphere-determined blood flows of less than 100%, 100–200%, 201–300%, and greater than 300% of control (nonischemic) blood flow.

201TI, the apparent “fill-in” to an ischemic zone would occur at a substantially slower rate with 99mTc-MIBI. In the present study in the resting anesthetized canine model, the substantial uptake in skeletal muscle is not present, and higher blood levels are anticipated, likely accounting for the redistribution phenomenon shown.

Group 2

When 99mTc-MIBI and 201TI are given 5 minutes after onset of reflow with sacrifice 5 minutes later, a positive linear correlation with blood flow is shown (Figure 4). In this case, flows are largely hyperemic. Myocardial tracer content of both agents in the reperfused zone is substantially lower than anticipated on the basis of flow measurements. This is consistent with reduced extraction efficiency at higher flow rates as shown previously for 201TI.31–34

Group 3

When 99mTc-MIBI and 201TI are given 5 minutes after reflow with sacrifice 30 minutes after administra-
tion, a negative linear correlation with blood flow is observed (Figure 5). The flow range determined 5 minutes after reflow is comparable to group 2. Of note, 99mTc-MIBI and 201TI activities approximate control levels with flows near control levels. Regions with flows less than control (less than 100%) show additional uptake of 201TI and 99mTc-MIBI between 5 and 35 minutes after reflow (Figures 4 and 5). Regions with modestly increased flow (100–200% of control) show decreases in activity relative to control after 35 minutes of reflow, suggesting only minimal insult to this zone. With increasing hyperemia, 99mTc-MIBI activity, like 201TI activity, decreases indicating a more rapid washout than in normal myocardium.33–35 Such washout is not observed in normal myocardium, even under conditions of hyperemia.36

Acknowledgments

The technical assistance of Ms. Patty Bischoff and the secretarial assistance of Ms. Jo Stanley and Ms. Riva Morgan are very much appreciated.

References

29. Piwnica-Worms DR, Kronauge JF, Holman BL, Marsh JD, Jones AG: Mitochondrial membrane potential affects uptake of hexakis(carboxymethylisopropylisonitrile) into cultured heart cells (abstract). Circulation 1988;78(suppl II):II-385
30. Liu P, Dawood F, Riley R, Houle S, McAulughlin PR: Could the myocardial tracer Te-MIBI be made to redistribute by altering its blood concentration (abstract)? Circulation 1988;78(suppl II):II-387

KEY WORDS: reperfusion • technetium 99m–MIBI • ischemia • thallium 201
Relations of the myocardial imaging agents 99mTc-MIBI and 201T1 to myocardial blood flow in a canine model of myocardial ischemic insult.
R C Canby, S Silber and G M Pohost

Circulation. 1990;81:289-296
doi: 10.1161/01.CIR.81.1.289

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/81/1/289

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/