Are the Kinetics of Technetium-99m Methoxyisobutyl Isonitrile Affected by Cell Metabolism and Viability?

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To investigate the role of cell viability and metabolism on the myocardial kinetics of a new tracer, technetium-99m-methoxyisobutyl isonitrile (Tc-99m-MIBI), 250 μCi/1 Tc-99m-MIBI was infused in isolated rat hearts under constant flow conditions. The hearts were studied after inducing irreversible damage by cytochrome c oxidase inhibitor sodium cyanide (n=8) or sarcolemmal membrane detergent Triton X-100 (n=8). The control hearts (n=6) received no toxins. Mean Tc-99m-MIBI peak accumulation activity was significantly reduced after cyanide (51.1±44.2% of control, p<0.01) and Triton (13.8±2.7% of control, p<0.001) administration. Kinetic studies also showed marked reduction in accumulation rates and marked increase in clearance rates for cyanide (p<0.01) and Triton (p<0.01) groups compared with controls. Potential changes in regional flow distribution were assessed using microspheres. When peak accumulation activity was corrected for these changes, there remained significant differences between the groups. In the cyanide and Triton groups, irreversible cell injury was confirmed by creatine kinase and lactate dehydrogenase release, triphenyl tetrazodium chloride staining, and electron microscopy. All the cells were viable in the control group. We conclude that the accumulation and clearance kinetics of Tc-99m-MIBI are significantly affected by cell viability. Tc-99m-MIBI kinetics appear to be dependent on sarcolemmal integrity and to a lesser extent on aerobic metabolism. (Circulation 1990;82:1802–1814)

Technetium-99m-methoxyisobutyl isonitrile (Tc-99m-MIBI) is a new synthetic lipophilic, cationic, myocardial perfusion tracer, and it represents a new class of cardiac perfusion agents. It has a first-pass myocardial extraction fraction of 65%1 (38–57% average peak extraction fraction2,3) and is avidly retained by the myocytes.1–11 Its clinical efficacy as a perfusion tracer is similar to that of thallium-201.12–20 Compared with thallium-201, Tc-99m-MIBI has the advantages of higher emission energy with less attenuation, shorter half-life with a higher permitted dose leading to greater resolution, and a longer residence time functionally resembling microspheres.1,4–6,12,13,21

Tc-99m-MIBI accumulates linearly in the myocardium according to blood flow in much the same manner as microspheres.1,4–10 It exhibits slow myocardial clearance and shows little redistribution with time.1–11 Tc-99m-MIBI has the potential for assessing the efficacy of interventions such as thrombolysis and reperfusion and the diagnosis and prognosis of various coronary syndromes.21–39 However, whether MIBI kinetics are affected by myocardial viability is still unresolved.7,21,34–51 This issue has important implications in understanding kinetic mechanisms of cations in the myocyte and in determining the clinical usefulness of these agents. Results from previous experiments have been difficult to interpret because of the inability to dissociate the contribution of flow from that of viability in the ischemic and infarcted regions of various models.

Therefore, we performed a series of experiments designed to determine the role of irreversible cell injury on Tc-99m-MIBI kinetics in an isolated rat heart model independent of significant flow limitations. Irreversible cellular injury was induced by either an inhibitor of aerobic metabolism, sodium cyanide,52–57 or a membrane detergent, Triton X-100,58–63

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Methods

The study was designed to compare the kinetics of Tc-99m-MIBI tracer accumulation and clearance in a constant-flow Langendorff preparation under conditions of control with standard Krebs-Henseleit buffer infusion, sodium cyanide infusion, or Triton X-100 infusion.

Experimental Preparation

All hearts were perfused with Krebs-Henseleit buffer prepared in the standard manner (mmol/l): 1.2 KH₂PO₄, 1.2 MgSO₄, 1.75 CaCl₂, 4.7 KCl, 0.5 EDTA, 118.0 NaCl, 25.0 NaHCO₃, and 11.0 dextrose. This was continuously bubbled with 95% O₂-5% CO₂ at a rate of 3 l/min to maintain pH at 7.4±0.05 and pO₂ at 500±50 mm Hg. The cyanide perfusate was prepared by dissolving 980.2 mg sodium cyanide in 2 l Krebs-Henseleit buffer to make a 10.0 mmol/l solution. Triton X-100 perfusate was prepared by adding 10 ml 100% Triton X-100 to 2 l Krebs-Henseleit buffer to make 0.5% solution of Triton X-100.

To study tracer accumulation kinetics, radioactive buffer was prepared by adding 500 μCi Tc-99m-MIBI to 2 l of the above described Krebs-Henseleit buffer to make a solution containing 250 μCi/l Tc-99m-MIBI.

Isolated, Perfused Heart Preparation

Male Sprague-Dawley rats (weighing 320–370 g, 3–4 months old) were anesthetized with 0.65 mg i.p. sodium pentobarbital. Simultaneously, 500 units heparin was administered. After deep anesthesia was achieved, the heart was removed through a rapid right parasternal thoracotomy. The heart was then immediately placed in Krebs-Henseleit buffer at 4°C. The aorta was identified, cleared, and connected to the perfusion apparatus. Throughout the experiment, the buffer was infused at a constant flow of 14 ml/min, which was controlled by a digital roller pump (Masterflex, Farmer Instruments, Burlington, Ill.). Constant temperature was maintained at 37°C by a coil-condenser heat-exchanger circulator connected to an MGW Lauda 20 waterbath. A warm saline-filled intraventricular latex balloon, inserted through the left atrial appendage, was used to continuously measure left ventricular developed pressure. The left ventricular developed pressure and aortic perfusion pressure were continuously recorded on a physiologic recorder (model 2800S, Gould Recorder, Cleveland, Ohio).

Tracer Kinetic Monitoring

The myocardial Tc-99m-MIBI activity was monitored during accumulation and clearance phases, at 10-second intervals with a collimated sodium iodide probe, placed 1 cm from the surface of the left ventricle. The output was interfaced with a discriminator, centered on the 140-keV peak, and the output was transferred to a multichannel analyzer (series 35, Canberra-Packard, Meriden, Conn.) where data were displayed. Data were then transferred to an IBM personal computer for storage and quantitative analysis.

Experimental Protocol

All hearts were initially perfused with Krebs-Henseleit buffer for 15 minutes to achieve stabilization. Thereafter, the hearts were divided into three groups.

Group 1 (control, n=6). Hearts were perfused for 30 minutes with radioactive Krebs-Henseleit buffer containing 250 μCi/l Tc-99m-MIBI to study the accumulation phase; then perfused with nonradioactive buffer (Krebs-Henseleit buffer without Tc-99m-MIBI) for 45 minutes to study the clearance phase. All flows were maintained at 14 ml/min. Buffers-perfused rat heart experiments generally use flows of 10–14 ml/min.7,8,64 We chose the upper end of this range to avoid potentially significant reductions in regional flow causing ischemia. However, this flow rate is still much less than the maximum coronary flow rate for the normal rat, which is in excess of 20 ml/min/g.65

Group 2 (cyanide, n=8). Hearts were first perfused with Krebs-Henseleit buffer with 10 mmol/l sodium cyanide for 30 minutes to achieve metabolic blockade. This perfusion was followed by the radioactive buffer perfusion for 30 minutes to study the accumulation phase, then the nonradioactive buffer infusion for 45 minutes to study the clearance phase. A subgroup of five hearts received an additional 5 mmol/l sodium cyanide for 30 minutes during the radioactive buffer infusion (accumulation phase) to determine the effect of prolonged exposure to cyanide infusion on tracer kinetics and the degree of myocardial injury. All hearts were perfused throughout the experiment at 14 ml/min regardless of their contractile response to sodium cyanide.

Group 3 (Triton, n=8). Hearts were perfused with Triton X-100 0.5% solution for 5 minutes. This was followed by the radioactive buffer infusion for 30 minutes to study the accumulation phase, then the nonradioactive buffer infusion for 45 minutes to study the clearance phase. As with the other experimental groups, the hearts were perfused at 14 ml/min regardless of their response to Triton X-100.

Assessment of Cellular Injury

Irreversible cell injury was confirmed by creatine kinase (CK) and lactate dehydrogenase (LDH) release, vital tissue staining, and electron microscopy. Cumulative CK and LDH concentrations were measured every 15 minutes from the effluent, using a Hitachi 737 analyzer as described previously.66–70 The overall total CK and LDH released was calculated by adding each 15-minute cumulative total for the first 90 minutes.

Triphenyl tetrazolium chloride (TTC) vital staining of tissue was used to detect the presence of dehydrogenase enzymes. Positive red color staining of the tissue indicated viability of the myocardium.71,72 The heart was sliced horizontally, parallel to the long axis, through the apex and placed in 50 ml TTC, which contained 12.5 mg/ml tetrazolium,
for 10 minutes. The positive staining pattern as a portion of the total myocardial cut surface was visually estimated. The heart staining was then categorized as normal, where all the myocardium was stained; absent, where no staining was visible; or partial, where only a portion of the myocardium was stained (all those in this category had between 25% and 75% of the total myocardium stained).

The other half of the heart was placed in universal fixative (4:1 gluteraldehyde:formalin), dehydrated in graded ethanol, and embedded in Epon/araldite epoxy resin according to standard techniques. Ultrathin sections were stained with saturated aqueous uranyl acetate and Reynold’s lead citrate and examined using a Philips EM 400 electron microscope. The interpretation of the electronmicrographs was performed by a cardiac pathologist unaware of the experimental status of the heart. In all, three of six hearts from the control group, four of eight from the cyanide group, and four of eight from the Triton group were examined by electron microscopy.

Assessment of Regional Myocardial Blood Flow by Microspheres

Although global flow was held constant in this model, significant alterations in regional myocardial blood flow to the endocardium could not be ruled out. Such alterations, if marked, can alter tracer delivery and correction would be necessary. Thus, a separate set of experiments was performed to assess regional blood flow.

With the same experimental preparation and model described above, 12 isolated rat hearts were divided into three groups: control (n=4), cyanide (n=4), and Triton (n=4). After the stabilization phase, each group received the respective toxins in the same dose as described above. At the end of the toxin infusion, or after the stabilization phase in the controls, 1.2 μCi tin-113–labeled microspheres (DuPont, Billerica, Mass.; 15.5±0.1-μm in diameter; half-life, 115.1 days; principal photons, 393 keV, 2.43×10¹⁰ spheres/μCi) were injected into the system during 20 seconds. The microsphere suspension was agitated for 20 minutes and was added to the system at 7.0 cm from the heart. Activity of several equal volume samples was well counted to ensure uniformity of dose, and the syringe activity was measured again after injection to confirm negligible residual activity.

The quantity of microspheres was chosen to ensure that at least 200 microspheres would be present in the smallest expected sample with normal blood flow, although evidence suggests that fewer microspheres are actually needed. The dose of microspheres was also chosen to ensure that the number of spheres per milligram would be far less than the maximum arteriolar density of 161.5 spheres/mg in the rat heart.

Two minutes after the microsphere injection, the hearts were removed from the apparatus. The right ventricle and atria of each heart were excised and discarded. The left ventricle was then transected into two rings. Each left ventricle ring was divided into four sections, and each section was divided into two segments (epicardial and endocardial). Each segment was weighed, and its activity was counted in a gamma counter (Autogamma 5650, United Technologies, Packard, Mississauga, Ontario).

Ethics

The study protocol was approved by the Committee on Animal Research at the Toronto General Hospital and was performed in accordance with the “Position of the American Heart Association on Research Animal Use,” adopted on November 11, 1984, by the American Heart Association.

Statistical Analysis

The high-resolution time-activity data collected by the multichannel analyzer were first subtracted for background and corrected for physical decay. Each accumulation phase curve and each clearance phase curve was then individually fitted to an exponential function to allow characterization of the rate constant. Numerical constants (K for accumulation, K’ for clearance) were established for each individual curve.

For the assessment of regional blood flow, an endocardial to epicardial ratio of total counts per milligram was established for each heart. Also, the mean of the endocardial fraction of the total flow was used as a correction factor for regional flow differences to the endocardium. Total Tc-99m-MIBI uptake was divided by this correction factor to calculate the corrected Tc-99m-MIBI uptake.

The average accumulation (K) and clearance (K’) constants, the means of total Tc-99m-MIBI uptake, CK and LDH release, endocardial to epicardial microsphere distribution ratio, and corrected Tc-99m-MIBI uptake were compared among the three experimental groups by analysis of variance, with intentional post-hoc subgroup testing between pairs of groups by the Newman-Keuls technique.

Results

Hemodynamic Recordings

Examples of hemodynamic recordings are shown in Figure 1. Continuous intraventricular pressure recordings showed that the mean systolic pressures at end of the stabilization period were 58.0±12.4, 54.8±12.8, and 53.9±12.0 mm Hg for the control, cyanide, and Triton groups, respectively. At the end of stabilization, all diastolic pressures remained between 0 and 10 mm Hg. In the control group, during the course of the entire experiment, a gradual rise in systolic pressure to 79.0±13.6 mm Hg was noted.

After cyanide administration, five hearts displayed a continuous increase in diastolic pressure and a decrease in pulse pressure. This led to myocardial arrest without any recovery despite continued main-
Toxin speed. In the example, infusion of cyanide showed marked further increase in diastolic pressure and fall in pulse pressure ending in myocardial arrest. Triton-X100 caused rapid fall in pulse pressure then arrest.

Tenance of flow. The other three hearts that received cyanide showed markedly reduced function with decreasing systolic and increasing diastolic pressures but never stopped contracting. After the 30-minute infusion of 10 mmol/l cyanide, these hearts showed minimal further recovery with diastolic pressures greater than 50 mm Hg. An additional 30-minute infusion of 5 mmol/l cyanide during the accumulation phase, used in one of these three hearts, did not alter this response.

In all the Triton-injured hearts, there was a rapid decrease in systolic pressure leading to myocardial arrest in less than 3 minutes without any recovery.

Tracer Kinetics Monitoring

Figure 2 shows examples of Tc-99m-MIBI accumulation and clearance from multichannel analyzer recordings. The control example shows practically linear accumulation and extremely slow linear clearance. These are identical to what we have observed previously in isolated heart models in which Tc-99m-MIBI kinetics were studied. For the cyanide-injured hearts, the peak accumulation was lower, and initial clearance was more rapid. This reduced peak accumulation and rapid clearance were even more striking after Triton-induced injury.

Peak accumulation activity represented as a percentage of control activity is shown in Figure 3A. Cyanide-injured hearts had a reduced peak activity at 51.1±44.2% that of control (p<0.05 for each group), and Triton-injured hearts showed a reduced peak activity to 13.8±2.7% of control (p<0.001 versus control, p<0.05 versus cyanide).

Curves reconstructed from the mean K constants of accumulation for each experimental group are shown in Figure 4. The constants are listed in the figures and are significantly different between each group. Cyanide-injured hearts had reduced accumulation compared with the control hearts. Triton caused even greater reduction in accumulation.

By assigning the individual peak accumulation a value of 1.0 for each group and by applying the mean K’ constants for clearance, proportional clearance curves were constructed (Figure 4). Clearance was distinctly more rapid in the cyanide and Triton groups than in the control group (p<0.05 for each versus control).

Assessment of Cellular Injury

Figure 5 shows the extent of irreversible myocardial cell injury based on total CK release for the first 90 minutes in each group. Cyanide caused almost a sevenfold increase in CK release compared with control (control mean CK, 44.6±7.9 IU; cyanide mean CK, 298.4±109.5 IU; p<0.01). Triton caused a sevenfold increase in CK release compared with that induced by cyanide (p<0.01). Triton caused a 45-fold increase compared with control (Triton mean CK, 2,030±379.8 IU; p<0.001).

The mean of total LDH released by control hearts was 6.9±4.2 IU. Cyanide induced almost a 30-fold increase in LDH release compared with control (cyanide mean LDH, 204.2±169.1 IU; p<0.01). The Triton-injured hearts released 65 times more LDH than did the control hearts (Triton mean LDH, 457.3±83.7 IU; p<0.01). Thus, cyanide and Triton

Figure 2. Typical time-activity curves as displayed on the multichannel analyzer. The x axis is time after the onset of Tc-99m-MIBI infusion. Uptake is 30 minutes and clearance is 45 minutes in duration. Note the linear uptake of Tc-99m-MIBI and its slow clearance in the control example compared with the reduced uptake and rapid clearance after cyanide and Triton X-100. MIBI, methoxyisobutyl isonitrile.
induced irreversible myocardial cell injury based on CK and LDH release, and Triton caused significantly more injury than did cyanide.

TTC staining was normal in all six control hearts. In five cyanide-injured hearts, TTC staining was totally absent. The other three hearts showed partial staining, ranging from 25–75% of the myocardium. All Triton-injured hearts had totally absent TTC staining.

According to electron microscopy, the control group showed completely normal architecture with occasional artifacts, likely related to processing. Cyanide-injured cells showed moderate mitochondrial changes that included swelling, irregular and disrupted cristae, and amorphous deposits. Contractile elements were generally preserved, although Z bands were not visible at a few foci. Sarcolemmal damage was evident in the form of focal membrane

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**FIGURE 3.** Bar graph of mean peak uptake activity for each group as a percentage of control (panel A) and mean uptake corrected for variations in endocardial blood flow (panel B). In both figures, the control group was assigned a value of 100%. MIBI, methoxyisobutyl isonitrile.

**FIGURE 4.** Time-activity curves for accumulation (top) and clearance (bottom). For accumulation, mean accumulation constant (K) is shown for each group. Cyanide- and Triton X-100–induced injury significantly alter accumulation. Triton has more significant effects than cyanide. For clearance, the y axis is the proportion of peak activity where the peak activity is given a value of 1.0. Clearance constant (K') is shown for each group. Triton- and cyanide-induced injury lead to rapid clearance. There is no difference in clearance after cyanide or Triton. MIBI, methoxyisobutyl isonitrile. *p<0.005 vs. control; **p<0.005 vs. cyanide.
disruption (Figure 6). Triton-injured cells showed much more extensive myocyte changes. Nearly all the mitochondria were enlarged and irregularly shaped, with marked loss of cristae. Concentric lamellar structures composed of electron-dense material were also seen; they had double membranes and were likely mitochondria as well. Z bands were irregular, sarcoplasmic reticulum and T-tubules were dilated, and there was marked intracellular edema. Nuclei showed chromatin clumping. However, the most significant change was the marked sarcolemmal disruption (Figure 7). The cell membrane, mitochondria, and nuclear changes seen are those described by others\textsuperscript{72,80,81} to represent irreversible cell injury.

**Assessment of Regional Microsphere Distribution**

The total blood flow during the experiment was held constant at 14 ml/min. However, the endocardial:epicardial ratios for microsphere uptake were as follows: controls, 1.19±0.14; cyanide group, 0.74±0.04 (p<0.05); and Triton group, 0.60±0.13 (p<0.05). The normal endocardial to epicardial microsphere distribution ratio for the rat heart in the intact animal has been shown to be 0.88±0.09.\textsuperscript{65} Thus, the endocardium showed a net reduction in flow with injury, and as a fraction, it represented 54% of the total flow in the control group, 42% of total flow in the cyanide group, and 38% in the Triton group.

After the microsphere redistribution was corrected for the endocardial fraction of the total flow, the peak accumulations of Tc-99m-MIBI, as a percentage of control, were 66±34% for the cyanide group (p<0.05) and 20±2% for the Triton group (p<0.01). There remains a significant reduction in Tc-99m-MIBI accumulation after cyanide and Triton (Figure 3B).

**Discussion**

The importance of flow in determining myocardial uptake and distribution of Tc-99m-MIBI as a perfu-
FIGURE 7. Electron micrograph of Triton X-100–injured cells, showing marked variation in mitochondrial size (thin arrow) (panel A). Most show a lamellar appearance, and some have tubular structures (thin arrow). No cristae could be seen. Inset: A subsarcolemmal bleb (thick arrow). Original magnification, ×1,500; inset, ×1,650. Electron micrograph after treatment with Triton, showing parts of a muscle fiber (panel B). There is sarcolemmal disruption (arrowhead) and separation of the sarcolemma from the cell contents. There is variation in size of the mitochondria and some show disruption of the cristae and an occasional one shows disruption of the outer membrane. Amorphous densities and some granular material is seen in some mitochondria. Original magnification, ×4,000.
sion tracer has been well established. MIBI distributes linearly according to myocardial blood flow as precisely demonstrated by microspheres.\textsuperscript{1,4–10} Clearance, on the other hand, is extremely slow. This is hypothesized to be due to binding of MIBI, after cell entry, to a membrane or cytosolic site.\textsuperscript{11}

The role of cellular metabolism and cell viability on the kinetics of MIBI and other cationic myocardial species is still controversial but has important implications in understanding the behavior of these tracers and properties of the myocyte.\textsuperscript{7,21,34–51} In addition, knowing the importance of metabolism and viability help to define the clinical applications of these tracers.

\textit{Clinical Studies on MIBI Related to Viability}

Wackers et al\textsuperscript{22} showed the potential role of Tc-99m-MIBI in diagnosing reperfusion and salvaged myocardium. In most patients with reperfusion, serial reductions in defect size were noted even after reperfusion was established. Were these changes due to local improvements in flow or changes in cell metabolism? Recently, Dilsizian et al\textsuperscript{37} showed a good correlation between rest Tc-99m-MIBI uptake and severity of coronary artery stenosis. The same investigators also showed a general correlation between Tc-99m-MIBI uptake and viability diagnosed by wall motion.\textsuperscript{38} However, they concluded that Tc-99m-MIBI is primarily a perfusion tracer and not a viability tracer. But, as Machac\textsuperscript{39} points out, either a perfusion tracer or a viability tracer would be expected to show the relation observed by these investigators. Clinical studies have, thus far, not been helpful in resolving the MIBI uptake and viability issue.

\textit{Other Models Relating MIBI Kinetics to Viability}

There is evidence that low-flow ischemia without infarction does not alter the relation of Tc-99m-MIBI uptake and blood flow.\textsuperscript{32,36} In dog models of short occlusions with reperfusion and stunning, Tc-99m-MIBI uptake is either normal\textsuperscript{36} or increased.\textsuperscript{8,34}

In the presence of myocardial infarction, various conclusions have been reported on Tc-99m-MIBI kinetics.\textsuperscript{21,35,40–42} In one such study by Freeman et al,\textsuperscript{40} thallium-201 uptake was found to be directly related to myocardial viability, but Tc-99m-MIBI uptake was also markedly reduced in the infarct zone. In a separate study, Freeman et al\textsuperscript{41} concluded that cell viability was necessary for Tc-99m-MIBI uptake. Sinusas et al\textsuperscript{35} reported that Tc-99m-MIBI activity was 25\% of normal in a reperfused infarct region where flow was restored to 86\% of normal, and they concluded that Tc-99m-MIBI reflects ischemic conditions and not just flow restoration. Furthermore, Verani et al\textsuperscript{41} noted that during occlusion Tc-99m-MIBI counts correlated well with flow rates, but during reperfusion, this correlation was poor, implying that uptake may also reflect cell metabolic integrity. In contrast, in a similar study with a longer occlusion time, Soufer et al\textsuperscript{42} reported Tc-99m-MIBI uptake was actually increased in the infarct zone relative to flow. Many of these models suffer from the problem of inability to clearly separate stunned from infarcted myocardium and the effect of variable flow during reperfusion.

The relation between Tc-99m-MIBI clearance and viability is also confusing. In a 2-hour occlusion model with reperfusion, Canby et al\textsuperscript{43} showed that Tc-99m-MIBI washout may be increased in the reperfused tissue similar to thallium-201. Although they attributed this to reduced extraction with increased flow, it can also relate to metabolic and viability changes in the risk zone. In an isolated rat heart preparation, Okada and Glover\textsuperscript{7} demonstrated that myocardial MIBI washout rate was significantly increased by ischemic flow rates, thus implying a metabolic-dependent component to MIBI retention. However, in an occlusion-reperfusion dog model, these same investigators\textsuperscript{44} reported that Tc-99m-MIBI's clearance was not altered by reperfusion or infarction. Here, they concluded clearance is not dependent on the metabolic state. However, the infarction group without reperfusion could have had reduced uptake on the basis of reduced flow. When examining a heavily flow-dependent tracer, clearance rate will vary with blood flow and tissue concentration. Therefore, these data cannot rule out the possibility that MIBI clearance is viability and metabolism dependent.

Although some of these studies do support the hypothesis that Tc-99m-MIBI kinetics depend on cell viability, conflicting data clearly exist. All of these studies had variable states of flow (ischemia, reperfusion, hyperemia, and no-reflow), all of which will influence Tc-99m-MIBI kinetics. In addition, there is a large admixture of metabolic states in the reperfused myocardium including normal, ischemic, stunned, and infarcted areas. The actual separation of the contribution of flow versus that of metabolism and viability on MIBI kinetics is thus difficult.

To address these deficiencies and conflicting information, we sought to study the effect of viability on Tc-99m-MIBI kinetics by using a model where global flow is held constant and where cell viability is altered in a controlled fashion, independent of flow. Although there were some variations in regional flow distribution, this did not alter our conclusions.

\textit{Tc-99m-MIBI Kinetics and Cell Metabolism}

Previous metabolic studies on Tc-99m-MIBI were confined to cell or whole heart culture models. In cultured chick heart cells, Kronauge et al\textsuperscript{45} showed that rotenone, an inhibitor of oxidative phosphorylation, did reduce MIBI uptake by 35\%, but there was no LDH release to indicate cell necrosis. In another study\textsuperscript{46} with cultured chick heart cells, mitochondrial protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP 5 mM/l) inhibited 72\% of MIBI uptake. Again, no LDH was measured. In contrast, Maublant et al\textsuperscript{47–49} showed that MIBI uptake was not inhibited by iodoacetate, ouabain, or 5 mM/l sodium cyanide in cultured newborn rat ventricular cells.
Recently, Ingwall et al.\textsuperscript{51} compared Tc-99m-MIBI kinetics to thallium-201 in cultured fetal mouse hearts injured by anoxia. Although the investigators conclude that thallium-201 may be more sensitive than Tc-99m-MIBI for indicating irreversible injury, their data suggest that Tc-99m-MIBI may be more specific. Overall, these studies support the concept that metabolism and viability can significantly affect Tc-99m-MIBI kinetics; however, the details differ depending on the model used.

**Cyanide-Induced Cell Injury**

It was the goal of our study to induce irreversible cell injury using biochemical means, without altering global flow. Sodium cyanide was an ideal agent to induce this injury because it inhibits cytochrome c oxidase, which is important in oxidative phosphorylation, thus preventing the production of ATP. Cyanide was recently shown to induce changes in ATP and calcium that are similar to no-flow ischemia.\textsuperscript{52} This makes it an ideal agent for assessing ischemic-like injury similar to ischemia without altering flow. Although some studies suggest that irreversible cell injury with cyanide can be induced in 2–12 hours, these studies used much lower concentrations of cyanide than we have used in our experiment.\textsuperscript{53–57}

**Detergent-Induced Cell Membrane Injury**

The other agent we used to induce cell injury was Triton X-100. This is a nonionic detergent of the Triton X series (polyoxyethylene p-t-octyl phenol). Compared with other detergents, it has a relatively high hydrophilic to lipophilic ratio and a relatively high critical micelle concentration value. This confers higher membrane affinity, making it a suitable agent for lysing cell membranes,\textsuperscript{58–63} whose disruption is still the hallmark of irreversible cell injury.\textsuperscript{71,72,80,81} Thus far, there have not been any studies of cationic tracer kinetics or binding characteristics in the presence of detergent-induced cell membrane disruption.

**Evidence of Cell Injury**

Cyanide and Triton X-100 induced irreversible cell injury based on CK and LDH release, TTC staining, and electron microscopy. Myocardial CK and LDH release are generally accepted as indicators of loss of membrane integrity and irreversible cell injury.\textsuperscript{40,45,51,53,80} With TTC staining, absence of staining implies enzyme release and, thus, loss of membrane integrity and cell viability.\textsuperscript{71,72} Electron microscopy has been used as the reference standard for evaluating cell injury. The hallmarks of irreversible injury include sarcolemmal disruption, mitochondrial swelling, distortion of cristae, and deposition of amorphous densities within the mitochondria.\textsuperscript{71,80,81} We have identified all of these changes.

Membrane lysis with Triton X-100 induced a greater amount of irreversible injury than did inhibition of cellular metabolism by cyanide. Compared with cyanide, Triton induced a sevenfold greater CK release, a 2.5-fold greater LDH release, and more mitochondrial and cellular destruction according to electron microscopy. These results are not surprising, for the rapid loss of membrane integrity induced by a membrane detergent would be expected to cause immediate irreversible injury. On the other hand, although aerobic metabolism inhibition would lead to a loss of energy supply and membrane integrity, other sources of energy such as that derived from glycolysis could be used. Such processes would retard the onset of irreversible injury.

**Regional Myocardial Blood Flow in a Nonflow-Limited Model of Injury**

Although global flow was held constant, we investigated the regional microsphere distribution and found there was a difference in endocardial to epicardial flow ratios between the groups. The control group ratio was slightly greater than that expected for normal rats of 0.89±0.09,\textsuperscript{65} and the cyanide group ratio was slightly less. When the peak accumulation of Tc-99m-MIBI was corrected for the different endocardial fractions of total flow, there was still a significant difference in accumulation between the irreversibly injured groups and controls. Thus, the effect of viability on Tc-99m-MIBI kinetics far exceeded the small differences in regional flow, further strengthening our conclusions.

**Tc-99m-MIBI Kinetics in a Nonflow-Limited Model of Injury**

Cyanide and Triton induced irreversible injury and altered MIBI kinetics. Tc-99m-MIBI accumulation is reduced in the irreversibly injured cell. In addition to the greater amount of injury, Triton was associated with a more dramatic reduction in accumulation than cyanide. These results support the direct relation between membrane integrity and Tc-99m-MIBI uptake and accumulation. The effect of altered cell energy metabolism on the uptake and accumulation is likely shown indirectly through effects on the membrane.

Clearance of MIBI was also markedly altered by irreversible injury. The usual avid retention of MIBI is no longer observed after irreversible injury, and Tc-99m-MIBI is rapidly cleared from the cell. This rapid clearance was seen with both Triton and cyanide but was not significantly different between these two groups. There are two possible explanations for this phenomenon. 1) MIBI clearance may be extremely sensitive to irreversible injury so that even mild degrees of membrane disruption by cyanide could have the maximum effect possible on clearance. Membrane disruption may result in the leakage of membrane-bound or cytosolic-binding sites\textsuperscript{11} for MIBI, or it may directly alter the binding characteristics of Tc-99m-MIBI to the membrane. Changes in cell environment resulting from membrane disruption could also alter potential binding sites. 2) Alternatively, inhibition of aerobic metabolism could itself affect the binding of Tc-99m-MIBI to its binding sites. Such binding may be an energy-dependent
process or may be sensitive to metabolic changes resulting from inhibition of aerobic metabolism (for example, acidosis, fatty acid metabolite build up, protein alterations, free radical formation, or ion concentration changes). Such changes would alter uptake and clearance of the MIBI cation.

Based on the results of our study and other available data in the literature, we propose the following model of Tc-99m-MIBI kinetics (Figure 8). In the presence of normal flow in viable myocardium, the uptake process occurs primarily by diffusion, driven by both electrical and concentration gradients. MIBI then binds to a cellular site in a process that is sensitive to the alterations in cellular aerobic metabolism. As long as the electrical and concentration gradients are maintained, this binding will take place. The potential for binding\textsuperscript{11} and the apparent volume of distribution\textsuperscript{2} of MIBI are so great that saturation of uptake does not occur in normal ranges for tracer delivery.\textsuperscript{6,10} Binding is also very avid so that little bidirectional MIBI exchange occurs, and thus, MIBI's myocardial clearance is extremely slow. However, with the loss of sarcolemmal integrity, there is loss of the driving force for uptake. In addition, irreversible membrane injury leads to alteration or leakage of potential binding sites. These changes reduce MIBI's cellular binding and facilitate its clearance from the myocardium. Thus, we observe less accumulation and more rapid clearance.

**Conclusions**

In conclusion, from our experimental data, Tc-99m-MIBI kinetics are significantly altered by irreversible cell injury. These cationic complexes are critically dependent on sarcolemmal integrity and to a lesser extent on aerobic metabolism.
Clinical Implications

The inability to separate slow flow and viability by other models, makes these results in a nonflow-limited model clinically relevant. The clinical implication is that an imaging defect on an MIBI scan may represent reduced flow or loss of cell viability with or without reduced flow. The corollary of this is that a normal MIBI scan represents normal flow and the presence of cell viability. If two scans were compared in which flow conditions were different in a viable region, a flow-enhancing effect would be noted. If the area was necrotic, then there would be an identical defect despite changes in flow conditions. In the assessment of reperfusion, MIBI is known to be able to distinguish failed from successful therapy. Our study further suggests that MIBI may also be able to detect myocardial viability.

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