

^{99m}Tc -sestamibi kinetics predict myocardial viability in a perfused rat heart model

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Abstract

Introduction ^{99m}Tc -sestamibi has been proposed as a viability imaging agent. The purposes of this study were: (1) to determine the relationship between myocardial viability and ^{99m}Tc -sestamibi kinetics using perfused rat heart models across a full spectrum of viability, (2) to do so under conditions where myocardial flow was controlled and held constant, and (3) to do so using multiple quantitative methods to assess myocardial viability.

Methods Twenty-three isolated rat hearts were perfused retrogradely with a modified Krebs-Henseleit (KH) solution. Four groups were studied: controls (C, $n=6$), stunned (S, $n=6$), ischemic-reperfused (IR, $n=6$), and calcium injured (CAL, $n=5$). Following a 20-min baseline and subsequent treatment phase, ^{99m}Tc -sestamibi was infused

over 60 min (uptake) followed by 60 min clearance. Treatment phases consisted of 20 min no flow for S, 60 min no flow followed by 60 min reflow for IR, and 10 min infusion of KH solution without calcium followed by 20 min infusion of KH solution with 2 times normal calcium for CAL hearts. Creatine kinase (CK) assay, triphenyl-tetrazolium chloride (TTC) staining, and transmission electron microscopic (TEM) analysis were used to determine tissue viability.

Results Myocardial peak ^{99m}Tc -sestamibi uptake (%id) was significantly decreased in IR (4.11 ± 0.22 SEM; $p<0.05$) and CAL (1.07 ± 0.13 ; $p<0.05$), but not in S (4.88 ± 0.17) as compared with C (5.99 ± 0.50). One hour fractional retention was $79.3\pm 1.9\%$ for C, $80.3\pm 1.3\%$ for S ($p=\text{n.s.}$), $79.1\pm 1.8\%$ for IR ($p=\text{n.s.}$), and $14.9\pm 4.3\%$ for CAL ($p<0.05$ compared to all other groups). ^{99m}Tc -sestamibi absolute retention (%id) 1 h after the end of tracer administration was significantly decreased in IR (3.26 ± 0.23) and CAL (0.15 ± 0.02) as compared with both S (3.92 ± 0.16) and C (4.52 ± 0.32) ($p<0.05$). CK increased significantly from baseline in the IR and CAL hearts. TTC determined percent viability was $100\pm 0\%$ for C, $98.3\pm 1.1\%$ for S, $82.8\pm 2.6\%$ for IR, and $0.0\pm 0\%$ for CAL. TEM analysis supported these findings. End tracer activity was significantly correlated with TTC determined percentage viable myocardium ($r=0.93$, $p<0.05$) and CK leak ($r=-0.90$, $p<0.05$).

Conclusion ^{99m}Tc -sestamibi myocardial activity is significantly reduced in areas of nonviability after 1 h of tracer uptake and 1 h of tracer clearance. There is a linear correlation between myocardial viability, as determined by three independent methods, and tracer activity.

Keywords ^{99m}Tc -sestamibi · Myocardium · Viability · Kinetics · Ischemia · Reperfusion

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Introduction

Myocardial viability is an important parameter to assess in patients with coronary artery disease. Patients presenting with acute myocardial infarction of varying durations need rapid assessment of myocardial viability to determine whether reperfusion strategies will still be effective in salvaging myocardial tissue. Patients with chronic coronary artery disease and poor left ventricular function require an accurate assessment of viability to determine whether percutaneous coronary interventions or coronary bypass surgery will help restore ventricular function and relieve chest pain. The morbidity and mortality following coronary bypass surgery may also depend on the amount of viable myocardium.

Myocardial imaging using positron emission tomography may be the standard for assessing myocardial viability, but is not widely available. Myocardial imaging using thallium-201 is more readily available and has been used extensively for assessing myocardial viability. However, because of the relatively fewer counts associated with thallium-201, viability imaging using ^{99m}Tc -labeled radiopharmaceuticals has been proposed. ^{99m}Tc -sestamibi is widely utilized for myocardial perfusion imaging in conjunction with exercise or pharmacologic stress and has also been proposed as a viability imaging agent. Several investigators have reported the utility of ^{99m}Tc -sestamibi for assessing myocardial viability [1–12], while a number of other investigators have questioned the utility of ^{99m}Tc -sestamibi for assessment of myocardial viability [13–22]. Furthermore, in several of these studies, the relative contributions of flow and viability to ^{99m}Tc -sestamibi uptake could not be determined. Thus, the purposes of the current study were: (1) to determine the relationship between myocardial viability and ^{99m}Tc -sestamibi kinetics using a number of perfused rat heart models across a full spectrum of viability, (2) to do so under conditions where myocardial flow was controlled and held constant, and (3) to do so using multiple quantitative methods to assess myocardial viability.

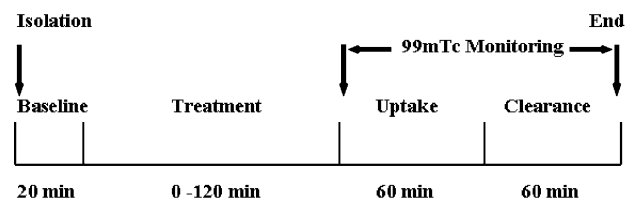
Methods

Isolated perfused rat heart preparation Male Sprague-Dawley rats (weighing 375–400 g) were used for this study. After deep anesthesia was achieved with 65 mg sodium pentobarbital, administered intraperitoneally, 400 units of heparin were administered intravenously. Rat hearts were rapidly excised from the chest and placed in ice-cold saline. The aortic stump was then immediately mounted by suture to the cannula of the perfusion apparatus and perfused in a retrograde manner at a normal flow rate

(12 ml/min) with a modified Krebs-Henseleit solution (mmol/l) of 1.25 KH_2PO_4 , 0.56 MgSO_4 , 1.51 CaCl_2 , 4.88 KCl , 0.833 ethylenediaminetetraacetate (EDTA), 127 NaCl , 20 NaHCO_3 , and 5.77 glucose. The solution was continuously bubbled with 95% O_2 /5% CO_2 to keep pH between 7.35 and 7.45, and O_2 at 250% saturation. The temperature of the perfusate was maintained at 37°C by water bath. A latex fluid-filled balloon was inserted through the left atrium into the left ventricle and connected to a Statham P23ID pressure transducer in order to measure left ventricular diastolic and systolic pressures. Coronary perfusion pressure was also monitored throughout each experiment using a similar pressure transducer connected to the perfusion line just before the cannula connected to the aortic stump. All hearts were atrially paced at 300 bpm throughout the protocol.

Study protocol Figure 1 illustrates the experimental design. After 20 min of baseline, hearts were subjected to the following treatments: control hearts ($n=6$) received no treatment and were perfused at 12 ml/min; stunned hearts ($n=6$) were treated with no flow for 20 min followed by reflow (5 min for hemodynamic stabilization); ischemic-reperfused hearts ($n=6$) were treated with 60 min no flow and 60 min reflow; and calcium injured hearts ($n=5$) were treated with 10 min of perfusion without calcium followed by 20 min perfusion with twice normal calcium concentration, followed by 10 min perfusion with normal calcium concentrations (for hemodynamic and calcium stabilization). Following treatment, 7.4 MBq (200 μCi) ^{99m}Tc -sestamibi was infused over 60 min (uptake). Hearts were then perfused with Krebs-Henseleit solution without tracer for 60 min (clearance).

Tracer monitoring Radiotracer activity was measured by a lead-collimated sodium iodide scintillation detector placed 3 cm from the heart and recorded over time by a computerized multichannel analyzer (MCA) (Canberra



Treatment:

Group 1: Control (no treatment, $n=6$)

Group 2: Stunned (20 min no flow, 5 min reflow, $n=6$)

Group 3: Ischemic-Reperfused60 (60 min no-flow, 60 min reflow, $n=6$)

Group 4: Calcium Injured (10 min no calcium, 20 min high calcium and 10 min normal calcium, $n=5$)

Fig. 1 Experimental protocol employed in this study. See text for details

Nuclear, Schaumburg, IL, USA) operating in multichannel scaling mode. Known activities of ^{99m}Tc were used to calibrate the recording system. ^{99m}Tc energy range was assessed by placing a source in the location that the heart would occupy, then operating the MCA in pulse height analyzer (PHA) mode. The detection window was set at 126–154 keV. Myocardial ^{99m}Tc -sestamibi activity was recorded at 60-s time intervals throughout the protocol. Time-activity curves during uptake and clearance were converted from analog to digital data and displayed on a computer monitor. Following each experiment, data were transferred to a spreadsheet for background subtraction, decay correction, and further analysis. Background activity was recorded prior to and at the end of each experiment. The end background activity was used for background subtraction. The end activities (μCi) for all hearts were measured with a dose calibrator. All hearts were weighed after activities were measured.

Triphenyltetrazolium chloride (TTC) Triphenyltetrazolium chloride (TTC) staining was used to determine myocardial tissue viability. At the end of each experiment, the great vessels, atria, and right ventricle of the heart were removed. The left ventricle was sectioned into four short-axis slices. Tissues were incubated in TTC solution with pH 7.8 at 37°C for 15 min. The TTC-stained tissue slices were photographed on both sides. These photographs were digitized by scanning into a computer and quantitatively analyzed using SigmaScan Pro software (Jandel, San Rafael, CA, USA). Digital planimetry was used to calculate the percentage of viable and nonviable myocardium in each slice by averaging the measurements of the TTC-positive and TTC-negative areas in photographs of the front and back of each slice and then summing these to derive a total for the left ventricle.

Others have also used TTC to assess for necrosis in a rat heart model using global ischemia [23].

Creatine kinase assay Creatine kinase (CK) release was measured over time to assess myocyte injury. A 1-ml sample of effluent from the perfused heart was collected every 15 min throughout the experiment and kept at 0°C. Using a spectrophotometer Model DU640 (Beckman, Fullerton, CA, USA) and CK assay kits (Sigma, St. Louis, MO, USA), CK sample concentrations were determined and corrected by heart weight (U/l per g).

Transmission electron microscopy (TEM) Krebs-Henseleit perfused hearts were subjected to transmission electron microscopy (TEM) for myocardial ultrastructural analysis. Two additional hearts representing each group were subjected to the same protocols stated above, but with no radiotracer administration, and were then subjected to TEM

analysis. When the experimental protocol was completed, each heart was perfusion-fixed with 0.2%, pH 7.4 buffered, glutaraldehyde (12 ml/min) for 5 min. Tissue samples were taken from the endocardial and epicardial regions of the left ventricle and further fixed by immersion for 1 h with 1% aqueous osmium tetroxide. Then, the tissue samples were en bloc stained overnight in 0.5% aqueous uranyl acetate, dehydrated by graded ethanol solutions and propylene oxide, and embedded in PolyBed 812. Two representative blocs were selected for ultrathin sectioning and examination in Zeiss 109 TEM. Ten grids per group were examined.

Data analysis After background subtraction and decay correction, myocardial ^{99m}Tc -sestamibi time-activity uptake data were plotted using the group mean counts from the multichannel analyzer. Myocardial clearance data were normalized to peak uptake counts. Absolute peak uptake activity was calculated by using the final well counter measured end activity and back calculating from the probe-determined clearance. Both the peak and end activities were corrected for injected dose of ^{99m}Tc -sestamibi (%id).

The data are expressed as mean \pm SEM. Between-group comparisons were performed by one-way analysis of variance. Post hoc comparisons were made using the *t*-test with Bonferroni correction in order to determine which of the groups were significantly different from controls. Probability values less than 0.05 were considered significant. Pearson *r* analysis was applied to assess the correlation between ^{99m}Tc -sestamibi myocardial retention and CK leak or TTC-determined percentage myocardial viability.

Ethics All experimental animals were handled in accordance with the guiding principles of the American Physiological Society and experimental protocols approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center.

Results

Hemodynamics The hemodynamic data are shown in Table 1. There were no significant changes in the control group during the experiment for any hemodynamic parameter. The stunned group demonstrated decreased left ventricular systolic and developed pressure. The ischemic-reperfused group demonstrated decreased left ventricular systolic and developed pressure, heart rate, and increased diastolic pressure. The calcium injured group demonstrated decreased left ventricular systolic and developed pressure, with increased coronary perfusion and left ventricular diastolic pressures. This group became asystolic despite pacing.

Table 1 Hemodynamic data (mean±SEM)

	Baseline	Treatment	30 min p tracer	60 min p tracer	90 min p tracer	120 min p tracer
CPP (mmHg)						
Control	52.5±0.85		52.3±0.9	52.7±0.8	53.2±0.8	53.0±0.7
Stunned	53.5±1.5		54.0±1.9	54.5±1.8	57.0±2.7	56.8±2.2
IR 60	50.7±0.7	48.1±1.6	48.7±2.3	50.3±2.8	53.0±2.5	54.3±2.3
Calcium	50.8±0.8	119.6±21.5*	153.6±19.9*	149.8±23.6*	148.4±24.6*	148.6±24.6*
LV systolic pressure (mmHg)						
Control	85.7±3.2		89.2±4.4	90.5±4.2	88.7±4.5	89.5±5.3
Stunned	90.5±2.6		75.0±2.6*	73.7±3.6*	70.2±3.5*	70.7±3.5*
IR 60	85.0±2.8	84.1±7.1	72.3±4.3*	73.3±5.8*	73.7±4.6*	74.0±4.4*
Calcium	84.6±2.3	69.8±9.4*	70.2±10.8*	67.8±12.1*	61.8±12.1*	62.2±11.9*
LV diastolic pressure (mmHg)						
Control	6.3±0.6		7.0±0.7	6.8±0.7	6.7±0.4	7.0±0.7
Stunned	6.5±0.6		7.8±1.0	6.5±0.9	5.5±0.9	5.3±0.7
IR 60	7.0±0.8	47.3±10.1*	44.0±11.4*	44.3±11.2*	43.7±12.2*	44.7±11.9*
Calcium	5.2±0.2	70.2±10.8*	68.8±9.4*	67.8±12.1*	61.8±12.1*	62.2±11.9*
LV developed pressure (mmHg)						
Control	79.3±0.6		82.2±3.9	83.7±3.6	82.0±4.4	82.5±5.3
Stunned	84.0±2.4		67.2±1.9*	67.2±2.9*	64.7±2.7*	65.3±2.9*
IR 60	78.0±2.9	37.7±10.6*	28.3±10.8*	29.0±11.5*	30.0±11.9*	29.3±11.7*
Calcium	79.4±2.2	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*
Heart rate (bpm)						
Control	300±0		300±0	300±0	300±0	300±0
Stunned	300±0		300±0	300±0	300±0	300±0
IR 60	300±0	270±30.0*	228±58.2*	232±58.5*	240±60*	240±60*
Calcium	300±0	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*	0.0±0.0*

CPP coronary perfusion pressure, LV left ventricular
 * $p < 0.05$ compared with baseline

Myocardial ^{99m}Tc-sestamibi kinetics

Myocardial ^{99m}Tc-sestamibi uptake Figure 2 shows ^{99m}Tc-sestamibi myocardial uptake kinetics (cpm/g) for the four groups. There was no significant difference in myocardial uptake between control and stunned groups. Both groups demonstrated linear uptake. Uptake in the ischemic-reperfused was significantly reduced ($p < 0.05$) compared to either the control or stunned groups. However, the uptake curve remained linear. The calcium injured group had the lowest uptake, which was significantly depressed relative to all other groups ($p < 0.05$).

Myocardial ^{99m}Tc-sestamibi fractional washout Decay-corrected and normalized ^{99m}Tc-sestamibi clearance curves for the four groups are shown in Fig. 3. ^{99m}Tc-sestamibi fractional retentions in the stunned and the ischemic-reperfused groups were not significantly different compared with the control group at any time. The calcium injured group demonstrated an accelerated clearance with a rapid early phase and a slower second phase. The fractional retention of ^{99m}Tc-sestamibi for the calcium injured group was significantly different from all other groups at all times

($p < 0.05$). One-hour fractional retention was 79.3±1.9% for control, 80.3±1.3% for stunned ($p = n.s.$), 79.1±1.8% for ischemic-reperfused ($p = n.s.$), and 14.9±4.3% for calcium injured hearts ($p < 0.05$ compared to all other groups).

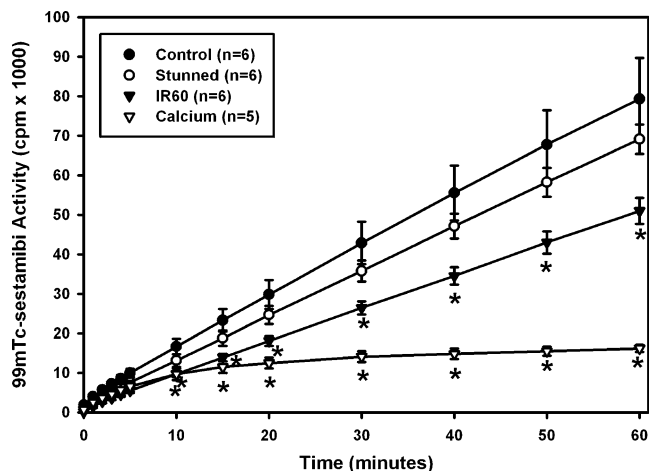


Fig. 2 ^{99m}Tc-sestamibi myocardial uptake time-activity curves. * $p < 0.05$ compared with control

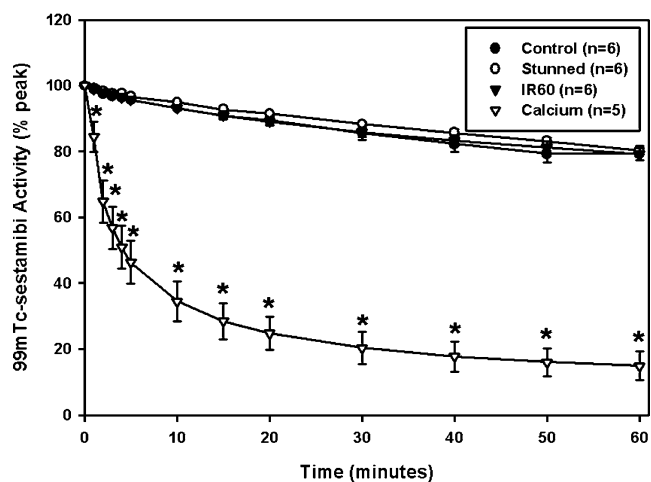


Fig. 3 ^{99m}Tc -sestamibi myocardial clearance time-activity curves. The curves have been normalized to 100% of peak activity at 60 min uptake. * $p < 0.05$ compared with control

Myocardial ^{99m}Tc -sestamibi peak and end activity Figure 4 illustrates peak and end ^{99m}Tc -sestamibi activities (%id). The peak activities in the control and stunned groups were 5.99 ± 0.50 and 4.88 ± 0.17 ($p = \text{n.s.}$). The ischemic-reperfused group had a significantly lower peak activity (4.11 ± 0.22) than either control or stunned groups ($p < 0.05$). Peak ^{99m}Tc -sestamibi activity in the calcium injured group was markedly and significantly decreased (1.07 ± 0.13) compared to all other groups ($p < 0.05$). The end activity for the control was 4.52 ± 0.32 , which was not significantly different from the stunned (3.92 ± 0.16 , $p = \text{n.s.}$), but was significantly higher than the ischemic-reperfused (3.26 ± 0.23 , $p < 0.05$) and calcium injured groups (0.15 ± 0.02 , $p < 0.05$).

Myocardial viability

CK assay The CK assay results are shown in Table 2. There was no significant difference in CK leak among the groups during baseline. No significant elevation of CK leak was found in the control and stunned groups during uptake and clearance in comparison with their respective baselines. In the ischemic-reperfused group, CK leak was significantly increased during ischemia and reperfusion and then returned toward baseline level during uptake and clearance. Calcium treatment induced CK leak in the calcium injured group significantly beyond baseline (approximately 50–100-fold). However, during uptake and clearance, CK leak returned to baseline level.

TTC analysis The percentage of viable myocardium, expressed as percent total left ventricle, was determined by TTC analysis. There was $100 \pm 0\%$ viable myocardium in controls, $98.3 \pm 1.1\%$ viable myocardium in the stunned

($p = \text{n.s.}$), $55.2 \pm 2.5\%$ viable myocardium in the ischemic-reperfused ($p < 0.05$ compared to control), and $0 \pm 0\%$ viable myocardium in the calcium injured group ($p < 0.05$ compared to control).

Transmission electron microscopy Representative TEM photomicrographs were made of thin sections of myocardial tissue samples for all four groups. The control (Fig. 5a) and stunned hearts (Fig. 5b) had normal morphologic appearance. In contrast, the ischemic-reperfused hearts exhibited mitochondrial abnormalities and focal areas of irreversibly injured cells (Fig. 5c). The calcium injured hearts exhibited evidence of severe, irreversible injury (Fig. 5d).

Correlation of myocardial viability with ^{99m}Tc -sestamibi activity A significant correlation between myocardial ^{99m}Tc -sestamibi end activity and peak CK leak (Log10 value) was obtained ($r = -0.90$, $p < 0.01$). The best fit to the data was a linear one as shown in Fig. 6. A positive correlation between ^{99m}Tc -sestamibi end activity and viability determined by percent TTC left ventricular staining was also significant ($r = 0.93$, $p < 0.01$). The best fit to the data was a linear one as shown in Fig. 7.

Discussion

^{99m}Tc -sestamibi myocardial uptake is primarily in mitochondria [24]. Cellular uptake and retention are dependent upon both mitochondrial and plasma membrane potentials [25]. Furthermore, calcium has been found to release ^{99m}Tc -sestamibi from the mitochondrial fraction. Since myocardial injury results in decreased membrane potentials and cellular and mitochondrial calcium overload, ^{99m}Tc -sestamibi has been proposed as a viability imaging agent.

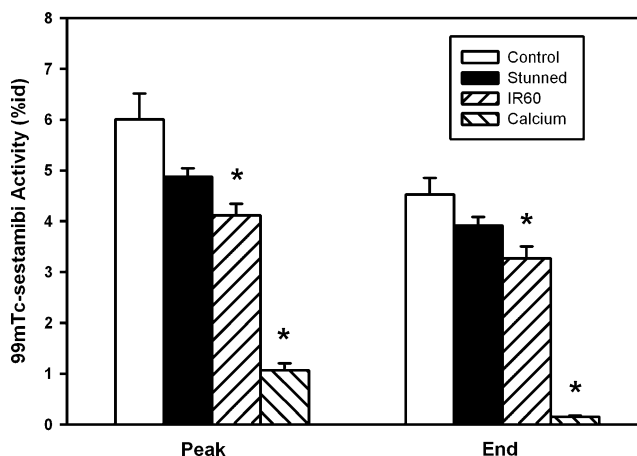


Fig. 4 ^{99m}Tc -sestamibi myocardial activity (%id) at peak uptake and at the end of 60-min clearance phase. * $p < 0.05$ compared with control

Table 2 Creatine kinase assay (U/l per g, mean±SEM)

	Baseline	Treatment 1	Treatment 2	30 min p tracer	60 min p tracer	90 min p tracer	120 min p tracer
Control (<i>n</i> =6)	6.3±1.7			5.5±0.9	4.3±0.7	6.4±1.2	5.0±0.9
Stunned (<i>n</i> =6)	10.4±1.5			4.5±0.8	3.3±0.8	4.8±0.9	6.0±1.4
IR 60 (<i>n</i> =6)	8.9±0.7	39.8±4.2*	19.1±3.5*	14.1±2.0*	12.6±1.7	10.8±1.2	10.6±1.1
Calcium (<i>n</i> =5)	5.4±1.1	260.4±52.9*	522.3±56.3*	7.9±1.3	2.7±0.6	2.9±0.8	2.1±0.6

**p*<0.05 compared with baseline

A number of investigators have reported data supporting the use of ^{99m}Tc -sestamibi as a marker of myocardial viability in models ranging from cultured cells, to perfused hearts, large animal models, and patient studies [1–12]. Maublant et al. demonstrated a positive correlation of tracer uptake with myocardial viability in cultured cells [12]. Perfused rat heart studies have been performed using Triton X, cyanide, and absence of glucose to induce injury, again demonstrating decreased uptake and decreased retention in nonviable myocardium [3, 8]. Larger animal models using swine and dogs have studied ^{99m}Tc -sestamibi kinetics using gamma camera imaging and implantable radiation detector probes [1, 2, 4–7]. Sinusas et al. used a 3-h coronary occlusion dog model followed by reperfusion [2]. They found that myocardial activity was significantly reduced compared with reperfusion flow in necrotic tissue, reflecting myocardial viability more than the degree of reperfusion. Takehana et al. used up to a 3-h model of coronary occlusion followed by reperfusion through a residual stenosis [7]. ^{99m}Tc -sestamibi uptake was found to accurately determine viability despite reperfusion through a residual stenosis. Finally, patient studies comparing ^{99m}Tc -sestamibi uptake to either functional parameters or fluorine-18 fluorodeoxyglucose (FDG) positron emission tomography have demonstrated the ability of ^{99m}Tc -sestamibi to assess viability [9, 10]. It should be noted that in patient studies flow is uncontrolled, making interpretation of the data difficult.

However, a number of other investigators have questioned the utility of ^{99m}Tc -sestamibi for assessment of myocardial viability [13–22]. Once again, models have ranged from cultured cells to perfused hearts, large animal models, and patient studies. Piwnica-Worms et al. studied cultured chick embryo myocytes and created severe cell injury using metabolic inhibition with iodoacetate and rotenone [15]. ^{99m}Tc -sestamibi uptake actually increased above control initially before declining later. Weinstein et al. used a rabbit heart model of coronary occlusion followed by reperfusion and ^{99m}Tc -sestamibi autoradiographs [17]. They showed that the initial uptake reflects predominantly coronary blood flow, independent of myocardial viability. A number of reports using pig and dog models of coronary occlusion followed by reperfusion also

found a poor correlation of ^{99m}Tc -sestamibi uptake with viability [13, 14, 18, 19, 21]. Despite a 24-h model of coronary occlusion, Merhi et al. concluded that ^{99m}Tc -sestamibi distribution was not significantly influenced by cell death [16]. Redistribution has also been found in necrotic tissue [14]. However, it is unclear if the tracer was actively taken up, or simply trapped. Finally, patient studies have also suggested that ^{99m}Tc -sestamibi underestimates viability when compared to positron emission tomography using 18F-FDG [20, 22]. Other investigators have suggested that the ability of ^{99m}Tc -sestamibi to assess myocardial viability may be time dependent [26, 27].

In the present study, our models created a full spectrum of myocardial necrosis ranging from 0 to 100% viability. There was an excellent correlation between ^{99m}Tc -sestamibi end activity and TTC assessment of viability. There was an excellent correlation between ^{99m}Tc -sestamibi end activity and CK release. TEM analysis confirmed myocardial injury in models demonstrating reduced tracer uptake.

^{99m}Tc -sestamibi kinetics ^{99m}Tc -sestamibi uptake and retention were both near linear in the control group. Fractional myocardial retention after 1 h washout was 79.3%. The stunned group exhibited uptake and retention not significantly different from the control group, indicating that this brief ischemia with reperfusion did not alter sestamibi retention in mitochondria. This conclusion was confirmed by measurement of absolute peak and end myocardial ^{99m}Tc -sestamibi activities, which were not different for the control and stunned groups. This supports other reports that ^{99m}Tc -sestamibi kinetics alone are not useful for the detection of stunned myocardium [28]. However, when accompanied by an assessment of regional wall motion, demonstrating normal tracer kinetics with abnormal function, stunned myocardium can be identified.

^{99m}Tc -sestamibi uptake in the ischemic-reperfused group was significantly depressed relative to the control and stunned groups. Myocardial retention was normal when expressed as a percent of peak uptake. However, absolute end ^{99m}Tc -sestamibi activity for the ischemic-reperfused group was significantly less than for controls (1.50±0.13 versus 2.23±0.08). We interpret this to mean that the depressed uptake curve in these hearts was due to a

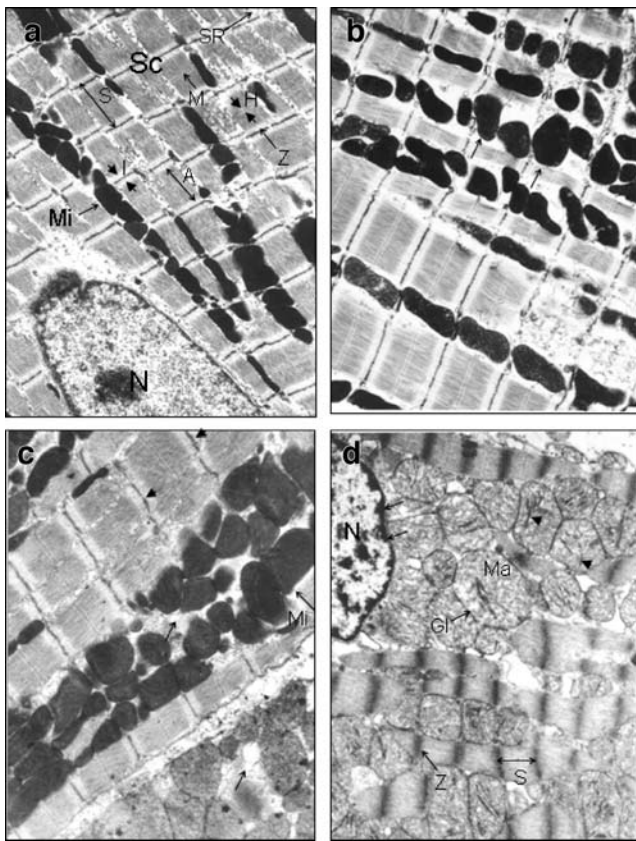


Fig. 5 **a** Rat myocardium from a control heart. The myofibers show an elongated nucleus (*N*) with normal structure. The sarcolemma (*Sc*), or cellular matrix, is mostly occupied by myofibrils showing cross striations specific to the contractile element: Z-band (*Z*), I-band (*I*), A-band (*A*), H-band (*H*), and M-band (*M*). The segment between two Z-bands is defined as a sarcomere (*S*). Mitochondria (*Mi*) are the most abundant organelles and are distributed in longitudinal rows between myofibrils. Also in the interfibrillar sarcoplasm are located elements of sarcoplasmic reticulum (*SR*). **b** Rat myocardium subjected to 20 min of no flow followed by reflow (stunned heart). Generally, the tissue exhibits normal structure, comparable to the controls. However, occasionally, hypertrophic, dense mitochondria are observed (*arrows*). **c** Rat myocardium subjected to 1 h no flow and 1 h reperfusion (ischemic-reperfused). The tissue exhibits focal, disintegrated structures. Regional abnormalities include: increased sarcoplasmic area and sarcoplasmic vacuolization (*arrow*). The contractile elements show abnormalities with thickening of Z-bands in some areas (*arrowhead*). The mitochondria are often swollen. **d** Rat myocardium subjected to 10 min no calcium and 20 min 2× normal calcium treatment (calcium injured). The tissue exhibits major disruptions. The myofibrils show reduced numbers of contractile elements and thickening of the Z-bands (*Z*). The nucleus has evident chromatin margination (*arrows*). The mitochondrial matrix (*Ma*) is lucent, the number of cristae is reduced, and glycogen granules (*Gl*) and paracrystalline inclusions (*arrowhead*) are present

combination of normal or mildly depressed uptake in viable cells and severely reduced uptake in nonviable cells. The normal retention curve in these hearts was likely due to a combination of normal retention in the viable cells that initially took up the tracer, with minimal contribution from the nonviable cells, which did not take up tracer.

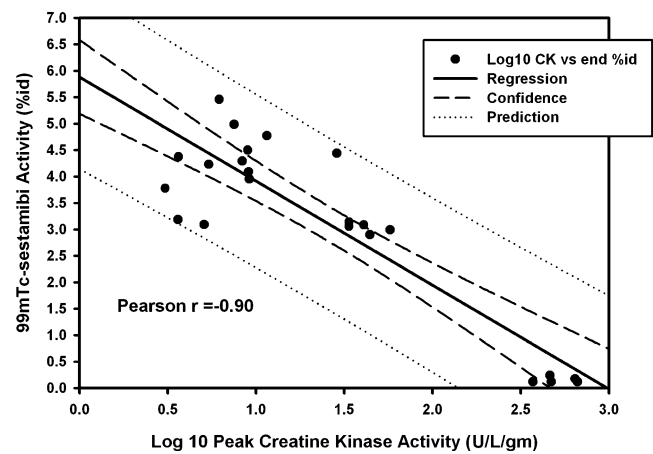


Fig. 6 Scatter plot illustrating the relationship between myocardial ^{99m}Tc -sestamibi end activity (%id) and peak CK leak (Log10 value)

Tracer uptake in the calcium injured hearts was very poor and appeared to approach an asymptote in these nonviable cells. Myocardial retention was also poor in the nonviable hearts. The calcium injured hearts had approximately 2% of the control activity remaining at the end of the experiments, which closely matched the measured TTC-assessed viability in this group.

Viability markers In the current study, we used three independent assessments of myocardial viability. CK leak was used as a quantitative measure to determine the extent of ongoing myocardial injury during the experiments. CK assays provided evidence that the control and stunned hearts were not injured during the experiment. The ischemic-reperfused group demonstrated a moderate rise in CK leak and the calcium injured group demonstrated a large increase in CK leak during treatment.

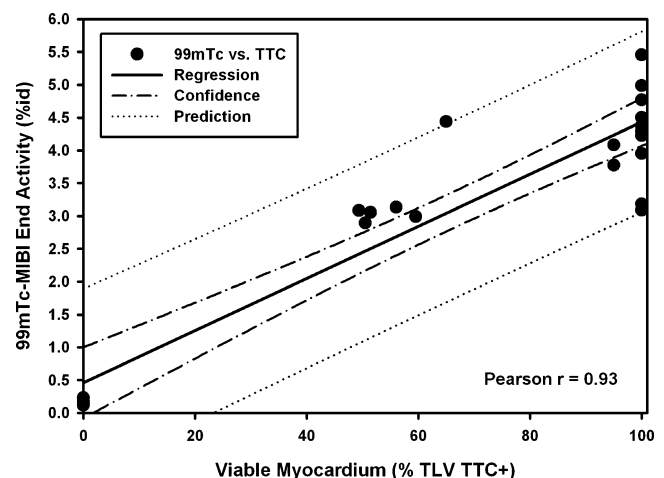


Fig. 7 Scatter plot illustrating the relationship between myocardial ^{99m}Tc -sestamibi end activity (%id) and viable myocardium detected by TTC staining

TTC staining and TEM analysis were used as measures of myocardial necrosis at the end of the experiments. TTC analysis demonstrated that the control and stunned myocardium were completely viable, the ischemic-reperfused myocardium was partially viable, and calcium injured myocardium was 100% nonviable. TEM analyses provided qualitative information regarding the ultrastructural morphology of cellular membranes and organelles. TEM revealed that the control and stunned hearts had normal appearance, while the ischemic-reperfused hearts demonstrated areas of focal injury. The calcium injured hearts were massively disrupted and demonstrated lucent mitochondria with glycogen inclusions, as well as membrane breaks, all indicators of loss of viability. Considered together, these data are consistent with mild insult to the stunned hearts, moderate injury in the ischemic-reperfused group, and severe injury in the calcium injured group.

Study limitations 1. The absence of tracer recirculation in this model allowed analysis of clearance without the complication of delayed uptake. However, it also precluded information regarding the effects of recirculation on clearance under the study conditions. ^{99m}Tc -sestamibi has been shown to demonstrate redistribution in certain situations, but only to a limited extent [28]. 2. Future studies using hibernating or apoptosis models and studies using autoradiography would be interesting. 3. Despite the results of this study, there still may be clinical difficulty differentiating viable but severely underperfused from necrotic myocardium. This difficulty may be minimized using nitrates during tracer injection. The current study was performed using a constant flow rate in order to eliminate this as a variable. However, future studies using low and high flows would be important. 4. This study utilized Krebs-Henseleit solution for perfusion. This perfusate has been extensively utilized by several laboratories. However, future studies should consider the use of a blood and protein perfusate as a more physiologic model.

Clinical implications Short- and long-term prognoses are strongly related to the degree of myocardial salvage and residual myocardial viability following reperfusion therapy in the setting of acute myocardial infarction. The current study demonstrates a strong correlation between ^{99m}Tc -sestamibi activity and viability. These results indicate that ^{99m}Tc -sestamibi patient imaging could accurately reflect the amount of myocardial salvage, and thus predict prognosis, almost immediately following reperfusion therapy with primary coronary interventions. This prediction could be made early after reperfusion when left ventricular function is still depressed due to stunning. Clinical imaging protocols based on these data would require further development. However, one possible protocol might in-

volve initial rest and 1-h delayed images, with the subsequent creation of a bull's-eye display based on clearance rates to assess and quantify viability.

Conclusion ^{99m}Tc -sestamibi myocardial activities are significantly reduced in areas of nonviability after 1 h of tracer uptake and 1 h of tracer clearance. There is an excellent linear correlation between the degree of viability and the degree of tracer activity. These results support the use of ^{99m}Tc -sestamibi imaging for assessing myocardial viability.

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