Early Scintigraphic Detection of Experimental Myocardial Infarction in Dogs with Technetium-99m-Glucaric Acid

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Recent data have generated some interest in technetium-99m (99mTc) glucaric acid as an in vivo viability marker. We studied 99mTc-glucaric acid retention in canine models of myocardial ischemia (20-min occlusion of the LAD/40-min reperfusion), acute myocardial infarction (MI) (90-min LAD occlusion/3-hr reperfusion), and chronic MI (90-min occlusion and either 48-hr or 10-day reperfusion). Regional myocardial blood flow was measured by radiolabeled microspheres. No preferential uptake of glucaric acid was observed in ischemic but viable myocardium. The compound showed high affinity for necrotic myocardial tissue for several days following injury. The preferential uptake in infarcted tissue disappeared by 10 days following injury. This study shows that 99mTc-glucaric acid acts exclusively as a marker of necrosis in canine models of MI. Technetium-99m-glucaric acid may have clinical utility in early cardiac imaging of myocardial infarction and in differentiating recent from old injuries.


Several technetium-99m (99mTc) complexes containing carbohydrate ligands have been reported as myocardial imaging agents. Both 99mTc-glucarate (1–3) and 99mTc-glucaric acid (4–6) have been used as myocardial infarction- (MI) avid agents. More recently, some interest has been placed on 99mTc-glucaric acid as a brain and heart agent. Preliminary studies in rat models of stroke and MI have indicated that 99mTc-glucaric acid may be preferentially retained in both necrotic and ischemic but viable tissue (7–9). The combined use of such an agent and a perfusion marker might yield clinically useful information on the presence and the extent of areas of ischemia that may be salvaged by therapeutic intervention.

However, the impact of these studies on 99mTc-glucaric acid is limited by some practical considerations. Substantial difficulties are encountered in rat models of disease when trying to distinguish between areas of viable and nonviable tissue. First, rodent models of myocardial and cerebrovascular disease are not ideal because the size of the organs does not allow optimal spatial resolution. Secondly, it is difficult, if not impossible, to achieve rat models yielding significant quantities of ischemic, viable tissue when obstructing nutrient blood flow for considerable time periods. This is due to the very limited degree of collateralization in the myocardial and cerebral circulation of this species.

It has been hypothesized that 99mTc-glucaric acid may act as a glucose analog, therefore preferentially retained in ischemic tissue where carbohydrate utilization is enhanced. Some preliminary data have indicated that insulin levels affect the biodistribution of this compound in rats (10). However, a higher 99mTc-glucaric acid heart retention in fasted animals after administration of insulin was also accompanied by significantly higher circulating blood levels of the compound.

We designed a series of experiments to evaluate the characteristics of 99mTc-glucaric acid retention in normal, ischemic and necrotic myocardial tissue over time. Canine models of myocardial ischemia and of ischemia/necrosis were employed.

METHODS

Glucaric Acid Labeling

The procedure developed to label D-glucaric acid was modeled on the components of a commercially available Glucoscan kit (E.I. du Pont de Nemours Co., No. Billerica, MA): 0.20 g Na(glucoheptonate) and 0.06 mg SnCl2·2H2O (11,12). The monocarboxylate salt of D-glucaric acid (0.2 g; Aldrich, St. Louis, MO) was reacted with one equivalent of aqueous NaOH in saline to form the dicarboxylate salt to which 0.1 ml of 3-5 mg/ml solution of SnCl2·2H2O in absolute ethanol was added. Approximately 30 min of reaction time following addition of 99mTcO4−-generated 99mTc-glucaric acid in high yield.
Qualitative characterization of the $^{99}$mTc complex was obtained by thin-layer chromatography (TLC) using Bakerflex® (Gelman Sciences, Ann Arbor, MI) SiO$_2$ strips and eluting with saline and methyl ethyl ketone (MEK). The TLC with saline showed all of the activity moving to near the solvent front, while with MEK all the activity stayed near the origin. Any TcO$_2$ colloid present would appear at the origin in the saline chromatogram, and unreacted pertechnetate would appear at the solvent front in the MEK chromatogram. Thus the extent of “labeling” can be ascertained rapidly (13).

**Animal Distribution Studies**

The in vivo distribution of $^{99}$mTc-glucaric acid was studied over a 60-min period of time in guinea pigs following standard procedures previously described (14). The animals were not food deprived before the experiment.

**Large Animal Surgical Preparation**

Studies were performed in 14 adult, male mongrel dogs (10-15 kg). The animals were fasted overnight and anesthetized with an i.v. injection of sodium pentobarbital (30 mg/kg). The dogs were then intubated and mechanically ventilated with room air (Harvard respirator). Animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

A 4F model PC-340 pressure transducer (Miller Instruments Inc., Houston, TX) was inserted into a superficial femoral artery through a small incision in the groin for measurement of arterial blood pressure. A 5F polyethylene catheter was placed in the contralateral superficial femoral artery and guided to the aortic arch for arterial blood sampling. A femoral vein was isolated and cannulated with a 5F catheter for the administration of medications, fluids, and $^{99}$mTc-glucaric acid.

Arterial blood samples were obtained at regular intervals throughout the experiments to monitor pH, PCO$_2$, and PO$_2$ (Model 178, Corning, Medfield, MA), and the respirator was adjusted to maintain arterial blood gases within the normal physiologic range.

With the animal in right lateral recumbency a left fifth thoracotomy was performed under sterile conditions, the lungs retracted, and the pericardium opened. A polyethylene tube was placed in the left atrial appendage for injection of radio-labeled microspheres. The left anterior descending (LAD) coronary artery was isolated for later placement of a snare for coronary artery occlusion. Myocardial blood flow was determined by the radioactive microsphere technique (15). Microspheres, 10-15 microns in diameter (E.I. du Pont, Boston, MA) labeled with either scandium-46, niobium-95, or tin-113 ($^{113}$Sn) were used. The microsphere suspension was injected as a bolus over 5 to 10 sec into the left atrium, after which the injection catheter was flushed with 10-ml of 0.9% saline. A 6-ml arterial blood sample was taken from the aortic arch catheter for 1 min following the initiation of the injection using a Sage model 351 infusion/withdrawal pump (Sage Instruments, Cambridge, MA).

**Postmortem Myocardial Analysis**

At the end of the experiment, the animals were killed by i.v. injection of T61-euthanasia solution (American Hoechst Co., Somerville, NJ). The hearts were removed, rinsed, and prepared for analysis by a modification of the method of Marcus et al. (16). This method allows for a quantitative presentation of the instantaneous nutrient blood flow at the time of each microsphere injection. The heart was removed and perfused with a 2, 3, 5 triphenyltetrazolium chloride (TTC, Sigma) and 0.2 M Sorenson’s buffer solution. The left ventricle was separated from the remainder of the heart, trimmed of epicardial fat and vessels, and divided into four slices from base to apex. Each layer was then cut into transmural specimens, which were then divided into epicardial and endocardial samples weighing ~1 g each. A total of ~90-100 samples were obtained from each heart. Specimens and reference bloods were assayed for scandium, niobium, tin and technetium in a Packard Auto Gamma Scintillation Spectrometer (Packard Instruments Co., Sterling, VA). Myocardial blood flow was calculated with the equation: Qm = (Cm × 100 Qt)/Cr, where Qm = myocardial blood flow (ml/min), Cm = myocardial tissue counts (counts/min/gram), Qt = reference blood withdrawal rate (ml/min), and Cr = counts in the reference arterial sample (dpm). Flow (ml/min) per gram of myocardial tissue was obtained by dividing blood flow by the sample weight.

**Protocols**

**Myocardial Ischemia.** After achieving stable baseline conditions, a first set of microspheres was injected in five dogs to obtain control myocardial blood flow values. The LAD was then occluded for 20 min, during which a second set of microspheres was injected. The occluder was removed and the LAD reperfused. To blunt the physiologic hyperemic response that follows reperfusion, a critical stenosis, obtained by tying a snare around an 18-gauge needle, was left in place. A third set of microspheres and 15-20 mCi of $^{99}$mTc-glucaric acid were injected. The animals were killed 40 min after reperfusion, and the hearts were removed and sectioned for determination of myocardial blood flow and $^{99}$mTc-glucaric acid retention.

Two additional dogs underwent similar procedures, but without LAD reflow. In these animals, $^{99}$mTc-glucaric acid was administered 20 min after LAD occlusion.

**Myocardial Necrosis and Reperfusion.** Following baseline steady-state measurements, the LAD was occluded for 90 min, during which a set of microspheres was injected. The artery was then reperfused and $^{99}$mTc-glucaric acid was injected i.v. following 3 hr (n = 3), 48 hr (n = 3), and 10 days (n = 3) reperfusion. The animals were killed 40 min after $^{99}$mTc-glucaric acid administration.

**SPECT Imaging**

Forty minutes prior to imaging, 18-22 mCi of the $^{99}$mTc complex were administered via a saphenous vein.

Tomographic imaging studies were performed using a Picker International Digital Dyna Camera with linearity correction (Picker International, Inc., Highlands Hts., OH). SPECT data were collected into 128 × 128 matrices using angular increments of 3° over 360°. Total imaging time was ~30 min. Images were acquired and reconstructed using a Siemens MAXDELTAX computer system (Siemens Medical Systems, Hoffman Estates, IL), using Medicl version 7.0 and SPECT version 3.0 software. A Butterworth filter of order 5 with a cutoff of 0.75 times the Nyquist frequency multiplied by a ramp was used as the reconstruction filter. Neither inter-
slice averaging, post-reconstruction filtering, attenuation correction, nor scatter correction was used. Conventional quality control as provided by the manufacturer was employed for center of rotation and uniformity correction. Uniformity acquisitions were obtained with 120 million counts.

Statistical Analysis
All computations were performed on an Apple Macintosh IIX personal computer using a commercially available statistical package (Statview II®, Abacus Concepts, Berkeley, CA). Data are expressed as mean ± 1 s.d.. The null hypothesis was rejected for p < 0.05.

RESULTS
Biodistribution
Very low heart uptake was demonstrated for 99mTc-glucaric acid in the guinea pig biodistribution studies. The compound is rapidly cleared from the blood via the urinary system (Fig. 1). Sixty minutes after administration 37.8% ± 1.59% of the injected dose is localized in the urinary bladder.

Table 1 summarizes the distribution of 99mTc-glucaric acid in various tissues 60 min after the administration of the compound.

Myocardial Ischemia
LAD reperfusion through a critical stenosis allowed the blood flow to return to ~90% of baseline values. Therefore, perfusion was restored, but the physiologic reactive hyperemic response was blunted. This precaution was assumed important to avoid possible flow-dependent increases in uptake.

In our canine model of acute myocardial ischemia, uniformly low retention of 99mTc-glucaric acid was demonstrated in both normally perfused and ischemic myocardial regions. Figure 2 shows the relationship between 99mTc-glucaric acid uptake, expressed as percentage of the injected dose, and blood flow distribution during LAD occlusion in the three dogs undergoing coronary occlusion and reperfusion. No changes in tracer uptake were visible in regions made ischemic by blood flow reduction. Superimposable results were also obtained in the two animals with no coronary artery reperfusion.

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<th>TABLE 1</th>
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<td><strong>Biodistribution of 99mTc-Glucaric Acid in Guinea Pigs 60 min After i.v. Administration (n = 3)</strong></td>
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Acute Myocardial Infarction
Following 90 min occlusion and 3 hr reperfusion of the LAD a large, transmural myocardial infarction was evident by TTC staining in all animals. The relationship between myocardial uptake of 99mTc-glucaric acid and blood flow during LAD occlusion is shown in Figure 3. An increased retention of 99mTc-glucaric acid in low flow regions was evident.

The myocardial samples with augmented retention of 99mTc-glucaric acid appeared to be necrotic by TTC staining (TTC−). A highly significant statistical difference (p < 0.0001; n = 3) was demonstrated between normal (TTC++; 0.006 ± 0.001 %ID/g), and both necrotic (TTC−; 0.019 ± 0.004 %ID/g) and mixed normal/necrotic (TTC±; 0.013 ± 0.003 %ID/g) myocardial samples (Fig. 4). Tracer uptake in necrotic and mixed normal/necrotic samples did not differ significantly. It is conceivable that ischemic but viable myocardial cells were present in the mixed normal/necrotic samples. However, their contribution to the 99mTc-glucaric acid increased uptake should be negligible on the basis of the results obtained in the models of pure ischemia.

![FIGURE 1](image1.png)

**FIGURE 1**
Distribution of 99mTc-glucaric acid in selected biologic samples over a 60-min period. A rapid blood clearance of the compound by the urinary system is evident. The data represent the mean ± s.d. of three animals per point.

![FIGURE 2](image2.png)

**FIGURE 2**
Relationship between myocardial uptake of 99mTc-glucaric acid and blood flow during LAD occlusion in a canine model of acute myocardial ischemia. A uniformly low uptake is evident in both normally perfused and hypoperfused myocardial regions.
FIGURE 3
Relationship between myocardial uptake of $^{99m}$Tc-glucaric acid and blood flow during LAD occlusion in a canine model of acute MI. Increased uptake of the compound in low-flow regions (necrotic by TTC staining) is evident.

Chronic Myocardial Infarction

48-hr Reperfusion. Preferential uptake of $^{99m}$Tc-glucaric acid in necrotic myocardial tissue was confirmed also in 48-hr-old myocardial infarcts (Fig. 5).

The uptake of the compound in necrotic (TTC−) myocardial tissue (0.029 ± 0.014 %ID/g) was significantly higher than in normal (TTC+; 0.002 ± 0.000 %ID/g; p < 0.0001) and in mixed normal/necrotic (TTC±) samples (0.01 ± 0.003 %ID/g; p < 0.002) (n = 3; Fig. 6). The retention of the compound in TTC± samples was also significantly higher in TTC+ regions (p < 0.0001).

Technetium-$^{99m}$Tc-glucaric acid retention in necrotic myocardial tissue was also significantly higher after 48-hr reperfusion than acutely after 3-hr reperfusion of the LAD (p < 0.01).

10-day Reperfusion. The animals killed after 90 min occlusion and 10 days reperfusion of the LAD showed the presence of scar tissue within the myocardium by postmortem visual examination of the heart sections. The uptake of $^{99m}$Tc-glucaric acid in infarcted regions was drastically reduced as compared with the two earlier time points. Uniformly low retention was demonstrated in all the myocardial samples obtained (Fig. 7).

SPECT Imaging

SPECT images obtained after 3 hr LAD occlusion and 24 hr reperfusion are presented in Figure 8. High $^{99m}$Tc-glucaric acid uptake is apparent in the anterior wall and in part of the septum in the distribution area of the LAD. Low background and bone uptake are also evident. Infarct localization and size were confirmed by postmortem TTC staining.

DISCUSSION

It has been postulated that $^{99m}$Tc-glucaric acid may be a marker of myocardial and cerebral viability (7–9). We demonstrated that this compound is not preferentially retained in vivo by ischemic but viable tissue in an occlusion/reperfusion canine model of myocardial ischemia. This animal model has been validated by several investigators both from the histologic and the metabolic point of view. However, it is known that substantial variations in the metabolic state of the myocardium may be observed in different models of the disease. Reperfusion may influence the status of the myocardial tissue by allowing the delivery of various blood-carried elements attracted to the ischemic areas by locally released attractants. Preliminary studies in a model of ischemia induced by LAD occlusion (but without reperfusion) also indicated the absence of any
preferential retention of $^{99m}$Tc-glucaric acid by ischemic tissue.

An enhanced retention of $^{99m}$Tc-glucaric acid in necrotic cerebral and myocardial tissue in rodents has also been demonstrated (7-9). We confirmed and expanded these data exploring the behavior of the compound at different time points following the induction of MI.

Technetium-$^{99m}$Tc-glucaric acid appears to mimic in several aspects that of $^{99m}$Tc-glucaric acid. Technetium-$^{99m}$Tc-glucaric acid is also preferentially retained by necrotic myocardial tissue acutely after and for several days following MI (6,17). This enhanced uptake disappears by 10 days after injury (18).

It has been demonstrated that mitochondria are the most important binding organelle for $^{99m}$Tc-glucaric acid in the intact heart cell (16). More specifically, cytochrome oxidase has been postulated being the $^{99m}$Tc-glucarionate-binding mitochondrial protein. It is possible that also $^{99m}$Tc-glucaric acid may bind to the mitochondria due to the structural similarities between the two compounds. The disruption of the cell membrane present during necrosis appears to be needed for the myocytes to take up these very hydrophilic complexes in high concentrations. The degree of "leakiness" produced by ischemia in our animal models was not sufficient to significantly modify the myocardial uptake of the compound.

The presence of MIs have been observed scintigraphically with several $^{99m}$Tc-labeled compounds, including $^{99m}$Tc-glucarionate (3), $^{99m}$Tc-glucaric acid (6), and $^{99m}$Tc-pyrophosphate (18). Technetium-$^{99m}$Tc-glucaric acid appears to be a suitable agent for early scintigraphic detection of necrotic myocardial cells. It has, for example, the advantage of favorable biodistribution characteristics. While $^{99m}$Tc-glucaric acid (20) are cleared from the circulation in dogs via the hepatobiliary system, $^{99m}$Tc-glucaric acid is rapidly cleared by the urinary system. The lack of a substantial accumulation of activity in the liver results in reduced potential interferences between this organ and the heart. An additional advantage of $^{99m}$Tc-glucaric acid is given by the very limited bone uptake. The presence of discrete amounts of activity in the bones, and more specifically in the ribs, have hindered the clinical use of both $^{99m}$Tc-glucaric acid and $^{99m}$Tc-pyrophosphate (21).

It has been demonstrated by Buja et al. (22) that $^{99m}$Tc-pyrophosphate uptake does not begin in the damaged myocardium until 12 hr after coronary occlusion in experimental animals. Twelve hours after the onset of symptoms also appears to be the earliest time for accurate detection of myocardial necrosis with $^{99m}$Tc-pyrophosphate imaging in humans (23). Uptake of $^{99m}$Tc-glucaric acid in necrotic tissue occurred in our animal model following just 90 min occlusion and 3 hr reperfusion of the LAD. This behavior may allow rapid and effective acute MI detection and sizing in the emergency room.

More recently, $^{111}$In-labeled anti-myosin antibodies have been employed for experimental and clinical myocardial infarct imaging (24,25). The results we obtained in experimental animals suggest that $^{99m}$Tc-glucaric acid may become a viable alternative also to indium-$^{111}$-anti-myosin antibodies. It may be preferable due to its low cost, rapid uptake and blood clearance, high image quality, and the lack of potential undesirable side effects.

The animal models of myocardial necrosis employed in our study were designed to maximize the extent of tissue injury by adding to 90 min of no flow ischemia the effects of variable periods of reperfusion. These models cannot be extrapolated to the majority of clinical situations, specially in the absence of thrombolytic therapy, where no coronary reflow is present. Additional studies should be performed to establish the clinical utility of this technique using protocols that do not include LAD reperfusion.

While not a viability marker, $^{99m}$Tc-glucaric acid appears to be an appropriate agent for early diagnosis...
The authors thank Dr. H.W. Strauss for invaluable discussions. These data were presented in part at the 37th Annual Meeting of the Society of Nuclear Medicine, Washington, D.C., June 1990.

REFERENCES