Quantification of myocardial injury produced by temporary coronary artery occlusion and reflow with technetium-99m–pyrophosphate

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ABSTRACT Previously, technetium-99m–stannous pyrophosphate (99mTc-PPi) has been used to localize and estimate the size of myocardial infarcts in animals after permanent coronary artery occlusion. This study tested the hypothesis that 99mTc-PPi accurately sizes myocardial infarctions produced by temporary coronary artery occlusion and reflow in dogs. Three groups of dogs were studied: group A underwent 3 hr of occlusion followed by 2 hr of reperfusion, with 99mTc-PPi injected 10 min after reflow (n = 10); group B underwent 3 hr of occlusion followed by 2 hr of reperfusion, with 99mTc-PPi injected 90 min after reflow (n = 11); and group C underwent 3 hr of occlusion followed by reflow with 99mTc-PPi injected at 10 min and again at 48 hr after reflow (n = 5). Myocardial slices from group A and B dogs were imaged in vitro. Group C dogs were imaged with single photon–emission computed tomography (SPECT) in vivo, and myocardial slices were imaged in vitro at the conclusion of the study. The extent of myocardial infarction was defined with triphenyltetrazolium chloride (TTC) staining, and coronary blood flow was estimated with radioactive microspheres. In addition, transmural myocardial tissue samples were taken from the center of the myocardial infarction, the lateral portion of the myocardial infarction, the normal myocardium adjacent to the lateral aspect of the infarcts, and from the normal myocardium and counted for 99mTc-PPi activity. A significant correlation was found between infarct size determined by areas of increased 99mTc-PPi uptake and that estimated from TTC staining for both group A (r = .89) and group B animals (r = .98). However, the intercepts and slopes relating estimates of infarct size from 99mTc-PPi uptake and TTC staining indicated that 99mTc-PPi overestimated size of infarction when injected early after reflow (group A), especially that of small infarcts (≤5 g). Results in group C dogs confirmed that, in the same dogs, estimates of infarct size were smaller when 99mTc-PPi was injected relatively late as compared with early after reperfusion. Thus, 99mTc-PPi injected 90 min or later after reperfusion provides an excellent means to identify and size myocardial infarcts produced by temporary coronary artery occlusion and reflow in this canine preparation. 99mTc-PPi uptake early, but not late, after reperfusion probably occurs in some myocardial cells that are severely injured but viable after reperfusion, and possibly in cells with excess cytosolic calcium that recover with longer periods of reperfusion.

mates of infarct size and with subsequent prognosis.7-9

Previously, we have found localization with 99mTc-PPi to be relatively specific for irreversibly injured myocardium after permanent coronary artery occlusion.10, 11 In animals with permanent coronary artery occlusions, 99mTc-PPi localizes in myocardial tissue with cell necrosis and multifocal mitochondrial calcification.11 However, other investigators have recently suggested that the extent of 99mTc-PPi uptake may overestimate infarct size after reflow because of its uptake by severely injured but viable myocardium.12, 13

Therefore, this study tested the hypothesis that 99mTc-PPi accurately localizes and estimates the extent of irreversibly damaged myocardium associated with temporary coronary artery occlusion followed by reperfusion.

Methods

Three protocols were used in this study: (1) 3 hr of temporary coronary artery occlusion followed by 2 hr of reflow, with 99mTc-PPi injected 10 min after reflow and postmortem imaging in vitro 2 hr after reflow (group A), (2) 3 hr of temporary coronary artery occlusion followed by 2 hr of reflow, with 99mTc-PPi injected 90 min after reflow and postmortem imaging in vitro 2 hr after reflow (group B), and (3) 3 hr of temporary occlusion followed by reflow, with 99mTc-PPi injected 10 min after reflow and tomographic imaging in vivo performed 2 hr after reflow, and with repeat injections of 99mTc-PPi after 48 hr of reflow with tomographic imaging performed 2 hr after injection followed by postmortem imaging in vitro (group C).

Protocol 1 (group A). Ten adult mongrel dogs were anesthetized with 30 mg/kg sodium pentobarbital and ventilated with room air with use of a Harvard respirator. A left thoracotomy was performed through the fifth intercostal space of each, and the heart was isolated in a pericardial cradle. Arterial catheters were placed in the left atrium and carotid artery for subsequent use in the determination of myocardial blood flow (MBF) by the reference flow method.15 Radioactive microspheres (15 μm) were injected into the left atrium to determine MBF under control conditions. The left anterior descending coronary artery (LAD) was occluded just distal to the first diagonal branch after the administration of 50 mg lidocaine. A second microsphere determination of MBF was obtained 1 hr after LAD occlusion. After 3 hr of LAD occlusion, reflow was established by removing the LAD occluder. After 5 min of reflow, a third batch of radioactive microspheres was injected to determine MBF. Ten minutes after reflow, 30 mCi of 99mTc-PPi was injected through the left atrial catheter.

After 2 hr of reflow, the animals were killed and the hearts removed. The hearts were placed in cold normal saline for 15 min to firm the tissue and facilitate slicing of the hearts from apex to base in 5 mm sections parallel to the atroventricular groove. The slices were then placed in a warm solution of 1% triphenyltetrazolium chloride (TTC) solution for 20 min and taken to the imaging facility.

The tissue sections were arranged, in order, on the surface of a high-resolution, parallel-hole collimator. Images were acquired until the total count from a single image, including all of the myocardial slices from a dog, was greater than 100,000 counts. Images were stored as a 256 × 256 × 10 digital matrix in the memory of a dedicated nuclear medicine computer (Technicare 500). Energy discrimination was provided by a 10% window centered on the 140 KeV photo peak of 99mTc.

After imaging was complete, transmural tissue samples approximately 2 to 3 mm in width were obtained from the following areas: (1) the infarct center (TTC-negative tissue), with the subendocardial section labeled A and the subepicardial section labeled B, (2) the lateral border of the infarct (TTC-negative tissue), with subendocardial portion labeled C and subepicardial portion labeled D, (3) areas of TTC-positive tissue lateral to sections described in 2 above, with the subendocardial section labeled E and the subepicardial section labeled F, and (4) areas of distantly normal tissue, with the subepicardial portion labeled G and the subepicardial portion labeled H. These tissue samples were weighed and placed in scintillation vials for determination of 99mTc-PPi activity by well counting.

Determination of infarct size with TTC. The cardiac slices were fixed in phosphate-buffered formalin and 35 mm color photographs were taken of both sides of each slice. The photographs were then traced to outline the total area of left ventricular tissue and an outline of the area of myocardial infarction (TTC-negative tissue). Each tracing was planimetered with a digitizing pad and the total left ventricular and infarct surface areas were determined. Atrial and right ventricular tissue was removed from the hearts and the weight of each left ventricular slice was determined and the thickness was measured. Infarct weights were determined by multiplying each slice weight by the ratio of infarct surface area to total surface area. Total infarct size was determined by summing the infarct weights for each slice.

Determination of infarct size with 99mTc-PPi. Sizing of myocardial infarction by 99mTc-PPi was accomplished by first determining the peak pixel density for all slices within the left ventricle. Then, for samples from animals in groups A and B, a threshold of 20% of the peak left ventricular pixel density was used to exclude areas of normal myocardium. This threshold was determined by examination of the ratio of pyrophosphate uptake by normal tissue (tissue sections E, F, G, and H) to that by infarcted tissue (tissue sections A and B) determined by 99mTc-PPi scintillation well counting. The ratio of 99mTc-PPi activity in the tissue sections inside the infarct (sections A and B) to activity in normal sections (sections E, F, G, and H) was at least 5.0 to 1.0, and thus a threshold of 20% was determined. The total number of pixels in each slice was determined, and the pixels from the area of infarction in each slice were multiplied by 0.0316 cm²/pixel to determine the surface area of infarction for each slice. This surface area of infarction was multiplied by the thickness of each slice and by 1.05 g/cm³ of muscle to determine infarct mass. The total infarct mass was determined by summing the infarct masses for all slices.

Tomographic images were obtained with the dogs positioned under the camera and right-side down. One hundred twenty projections were obtained over 360 degrees at a zoom of 1.4:1. A rotating, wide-field-of-view gamma camera (Technicare Omega 500) equipped with a low-energy, high-resolution, parallel-hole collimator was used for imaging. Projection images were acquired for a preset time of 10 sec and contained approximately 300,000 counts each. The radius of rotation was 20 cm and projection images were stored as 64 × 64 × 10 matrices. All projection images were corrected for field nonuniformity. Transverse sections of 2.7 mm thickness were reconstructed by filtered backprojection. The filter used for reconstruction was the product of a Butterworth low-pass filter with a frequency cutoff of 0.50 with an order of 6.0 and a ramp filter. Attenuation correction was performed. Voxels dimensions were determined by phantom studies and were 0.019 cm³/voxel.

The SPECT infarct sizing procedure required the localization of 99mTc-PPi uptake identified from the tomographic slices.
Noncardiac osseous activity was removed from the $^{99m}$Tc-PPi tomographic slices by masking around the areas of identified myocardial uptake of $^{99m}$Tc-PPi on a slice-by-slice basis. Myocardial uptake was determined from the activity remaining in the $^{99m}$Tc-PPi transaxial sections by a simple three-dimensional thresholding procedure at 20% of the peak voxel value within the infarct volume. The number of "infarct voxels" was then determined, and the size of the myocardial infarction was calculated by multiplying the infarct voxels by 0.019 cm$^3$/voxel and 1.05 g/cm$^3$ muscle weight.

**Protocol 2 (group B).** In protocol 2, 11 dogs were prepared and evaluated as described in protocol 1, except that they received an injection of $^{99m}$Tc-PPi 90 min after reflow. Otherwise, image processing, processing of the myocardial tissue, and determination of infarct size were performed as described for protocol 1.

**Protocol 3 (group C).** In protocol 3, the animal preparation was similar to that used in protocols 1 and 2 except sterile conditions were maintained during surgery. Also, a left atrial catheter was inserted for the injection of $^{99m}$Tc-PPi 48 hr after initiation of reflow. Each dog underwent 3 hr of LAD occlusion followed by 2 hr of reflow, with microsphere determinations of MBF as described in protocol 1. Thirty milliaccuries of $^{99m}$Tc-PPi was injected 10 min after reflow. The dogs' thoracotomies were closed approximately 30 min after reflow and they were taken to the imaging facility 2 hr after initiation of reflow. The animals underwent single photon-emission computed tomographic imaging as described above and were returned to the animal facility to recover for 48 hr. Approximately 48 hr after reflow, the animals were again injected with 30 mCi of $^{99m}$Tc-PPi and underwent imaging with SPECT 2 hr later. After SPECT imaging, the dogs were killed and imaging was performed in vitro as described in protocol 1.

**Radioactive microsphere estimates of MBF.** Radioactive microspheres ($^{46}$Sc, $^{85}$Sr, $^{99}$Nb, $^{57}$Co, $^{113}$Sn), 15 μm in diameter, were suspended in a sterile solution of Tween 20 and vigorously agitated in a Genie Vortex mixer. Two to six million microspheres were injected into the left atrium over 10 sec. Cardiac arterial blood was sampled at a constant rate of 7.75 ml/min beginning 10 sec before and continuing 90 sec after administration of microspheres. Regional MBF was calculated from sample radioactivity by the reference flow technique. The tissue samples obtained for $^{99m}$Tc-PPi well counting and larger tissue sections from normal plus ischemic tissue were submitted for microsphere determination of MBF.

**Statistical methods.** All three protocols involved statistical comparisons of TTC-determined estimates of the extent of myocardial infarction with those made by $^{99m}$Tc-PPi imaging. Regression equations were calculated by the least squares method. The correlation coefficients reported are Pearson's $r$ values calculated by regression analysis. Infarct sizes are reported as the group means ± SD.

**Results**

**Estimation of the size of infarcts.** The infarcts produced by 3 hr of coronary occlusion and reperfusion were characterized in TTC-stained slices by areas of necrotic unstained myocardium that occurred in the subendocardium and had variable extension into the subepicardium. The correlation between TTC and $^{99m}$Tc-PPi estimates of infarct size for group A dogs (injection 10 min after reflow) was good, with an $r$ value of .89 (figure 1), but the intercept was 5.8 g. Thus, relatively small infarcts (i.e., those $\leq 5$ g) were often overestimated by $^{99m}$Tc-PPi. Larger infarcts were sized more accurately when compared with TTC determinations. The correlation between TTC and $^{99m}$Tc-PPi estimates of infarct size for group B animals (injection at 90 min after reflow) was excellent ($r = .98$; figure 2). The intercept was 0.68 g and the slope was approximately 1.0. Thus, excellent estimates of all sizes of myocardial infarctions were obtained when $^{99m}$Tc-PPi was injected 90 min after reflow.

Group C dogs served as their own controls, and the infarct sizes determined by SPECT were larger in all five dogs when $^{99m}$Tc-PPi was injected 10 min after reflow as compared with when $^{99m}$Tc-PPi was injected...
TABLE 1

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>TTC staining (g)</th>
<th>In vitro 99mTc-PPi imaging</th>
<th>Early SPECT</th>
<th>Delayed SPECT</th>
</tr>
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<tr>
<td>23</td>
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<td>21.0</td>
<td>26.1</td>
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</table>

Mean ± SD 8.0 ± 8.6 9.6 ± 9.7 14.9 ± 10.7 8.8 ± 8.1

48 hr after reflow (table 1). The correlation between results with SPECT and those with TTC staining improved when 99mTc-PPi was injected after 48 hr of reflow as compared with when 99mTc-PPi was injected 10 min after reflow was established.

Regional MBF. The relationship between relative regional MBF and 99mTc-PPi uptake determined by tissue counting for all myocardial samples from group A dogs was an inverse exponential relationship with an r value of −.78 (figure 3, A). The relationship between 99mTc-PPi uptake and regional MBF was similar for group B dogs (figure 3, B), with an inverse exponential relationship (r = −.82). The relationship between 99mTc-PPi uptake ratios and regional blood flow for group A and B animals was similar (figure 4). In areas in which flow was severely reduced (0 to 10% of normal), 99mTc-PPi uptake was increased the most relative to that by normal tissue, whereas in regions with more moderate reductions in coronary blood flow, the relative 99mTc-PPi uptake was increased to a lesser extent.

In dogs in groups A and B, the well-counting results in subepicardial and subendocardial tissue samples taken from various regions within and adjacent to areas of infarct demonstrated sharp differences between relative 99mTc-PPi activity in areas of infarction and that in normal areas (figure 5). The mean background activity (corrected for injected dose and decay) in normal myocardium from group A dogs was 1,032,263 ± 415,414 counts/g and that in group B dogs was 1,909,414 ± 1,399,282 counts/g (p = NS). The ratio of 99mTc-PPi activity in the samples inside the infarct (samples A and B) compared with that in samples just outside the infarct (samples E and F) was at least 5.0:1.0 for dogs in groups A and B.

Discussion

Recent efforts to salvage ischemic myocardium in the early stages of acute myocardial infarction have focused on reperfusion therapy. The size of myocardial infarction is an important predictor of patient prognosis, and measurement of infarct size is important in the assessment of potential interventions to limit it. The clinical methods for measurement of infarct size after reperfusion therapy are still under development. 99mTc-PPi with SPECT provides an accurate means to size myocardial infarcts in patients who have not undergone reperfusion. Early reperfusion has been shown to allow earlier detection of infarction by 99mTc-PPi imaging, but the ability to size infarcts after early reperfusion has not been studied in detail. However, preliminary reports have suggested that the 99mTc-PPi technique may overestimate actual infarct size after reperfusion. Therefore, in this study, we investigated the relationship between 99mTc-PPi and TTC esti-
mates of infarct size in dogs with temporary coronary artery occlusions followed by reperfusion. Previous studies have demonstrated that TTC staining accurately detects necrosis after 3 to 6 hr of coronary occlusion.20, 21

Our results show that when $^{99m}$Tc-PPi is injected 10 min after reflow, infarct size is sometimes overestimated. This overestimation is confined primarily to infarcts of less than 5 g. When $^{99m}$Tc-PPi is injected 90 min after reflow, there is no systematic overestimation of infarct size. In fact, infarcts of all sizes are determined accurately when $^{99m}$Tc-PPi injections are delayed. The reperfusion preparation provides a model in which both severely ischemic and necrotic tissue are present. Thus, the results of this study suggest that severely ischemic cells may take up $^{99m}$Tc-PPi when it is injected within the first 10 to 15 min after reflow. However, when $^{99m}$Tc-PPi is injected 90 min after reflow, its uptake is limited to severely and apparently irreversibly damaged cells within the infarct area. Recent evidence has implicated calcium overload as a major factor contributing to reperfusion injury.32 Also, calcium antagonists, when given early after occlusion, can be protective and extend the time period for preservation of ventricular function during experimental temporary coronary occlusion.23, 24 We hypothesize that a significant change in calcium concentration occurs within severely ischemic cells between 10 and 90 min after reflow such that with immediate reflow there is a population of injured cells with increased calcium concentrations in which irreversible damage does not develop.

Several previous reports have described an overestimation of infarct size by $^{99m}$Tc-PPi in preparations of temporary coronary occlusions followed by reperfusion.12, 13, 25, 26 In these earlier studies, injections were usually made early after reflow. The phenomenon of $^{99m}$Tc-PPi uptake by severely ischemic but not necrotic cells appears to be confined to a very brief period early after reflow in this canine preparation. This uptake can

FIGURE 4. The relationship between mean ratio of test (infarct) tissue/normal tissue and percent normal flow by $^{99m}$Tc-PPi tissue counting for dogs in groups A and B.

FIGURE 5. The mean ratio of test tissue/normal tissue by $^{99m}$Tc-PPi tissue counting for various regions within and adjacent to the infarct. Region labels (A through F) are as defined in Methods. A, Data from protocol 1; B, data from protocol 2.
result in overestimation of infarct size when $^{99m}$Tc-PPi is injected sooner than 90 min after reperfusion. In the clinical setting, reflow established by thrombolysis is limited by residual coronary artery stenosis, and cellular metabolic function may be altered for a variable time period. Under such conditions, the relationship between pyrophosphate uptake and reflow may be altered, and further clinical investigation will be needed to study this relationship.

The reperfusion preparation also provides a means to study the relationship between $^{99m}$Tc-PPi uptake and severity of ischemic injury without the restriction of limited residual blood flow. Previous studies have found $^{99m}$Tc-PPi uptake to be related to regional blood flow and to the degree of cellular injury. A limitation of earlier studies was their use of permanent coronary occlusion, which resulted in very little residual blood flow to the center of the infarct. Therefore, delivery of the radiopharmaceutical to the most severely injured area was limited. With use of our preparation, there was no limitation of coronary blood flow, and we found an inverse exponential relationship between regional MBF during coronary occlusion (as a measure of severity of injury) and $^{99m}$Tc-PPi uptake after reflow. This inverse exponential relationship was observed for injections both early and late after reflow. A potential limitation of the present study is the simple edge-detection algorithm used in imaging in vitro and in vivo. SPECT imaging has more limited spatial resolution than the planar imaging in vitro used in protocol 1. Furthermore, no attenuation correction was attempted for SPECT imaging. However, future improvements in tomographic systems and in edge-detection algorithms should help eliminate these sources of error. A further potential limitation of this study relates to the injection of $^{99m}$Tc-PPi followed by killing of animals after 100 min in protocol 1 and injection followed by death after 30 min in protocol 2. This difference might have resulted in increased background in normal myocardium in group B dogs. However, our data demonstrate no statistical difference in the mean activity in normal myocardium in dogs in groups A and B. Furthermore, the threshold used was determined by examination of data from both protocol 1 and 2, and the same threshold was used for both protocols.

Rapid reperfusion after thrombolytic therapy or angioplasty may limit the usefulness of MB-creatine kinase curve analysis for the determination of infarct size. SPECT with $^{99m}$Tc-PPi imaging allows accurate localization and determination of infarct size when $^{99m}$Tc-PPi is injected 90 min or more after reflow. Furthermore, injection of $^{99m}$Tc-PPi early after reflow may identify infarcted cells and cells severely injured but viable after reperfusion therapy. Our data suggest that a subset of cells is potentially capable of recovery and may take up $^{99m}$Tc-PPi when it is injected early but will not when its administration is delayed until slightly later after reperfusion. Further studies are needed to define the metabolic changes responsible for this phenomenon.

In summary, this study shows that $^{99m}$Tc-PPi imaging can accurately size myocardial infarcts in dogs when the radiopharmaceutical is injected 90 min after reperfusion. These results suggest that $^{99m}$Tc-PPi with SPECT 90 min or more after initiation of reflow should be useful in the determination of size of infarction after reperfusion therapy.

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