Noninvasive Quantitation of Myocardial Infarction with Technetium 99m Pyrophosphate

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SUMMARY

We sought to quantitate infarct size using radioactive imaging techniques. Infarcts were created in closed chest dogs. Using a scintillation camera interfaced to a computer, infarct images were made in the anterior, left lateral, LAO, and RAO projections, 48 hours after infarction and 75 to 90 min following the intravenous injection of 15 mCi of Technetium 99m pyrophosphate (Tc-PYP). Images were computer enhanced and area was calibrated with a radioactive grid source of known dimensions. Image radioactivity was normalized for decay and dose corrected for body weight. Animals were sacrificed two hours following the injection of Tc-PYP. Postmortem images were also computer enhanced and calibrated. Gross infarct area and weight were estimated and transmural biopsies were evaluated for Tc-PYP activity and analyzed for creatine phosphokinase (CPK) content. Contiguous biopsies were pathologically analyzed and graded.

There was a negative correlation between tissue Tc-PYP activity and CPK content (r = -0.89). Pathologic severity worsened with increased Tc-PYP activity and diminished CPK content. There was a good correlation between gross infarct area and image infarct area, both in vivo (r = 0.79), and at postmortem examination (r = 0.95). Gross infarct weight also correlated well with image infarct activity in vivo (r = 0.83 in the RAO view) and at postmortem examination (r = 0.87). An additional correlation between gross infarct weight and in vivo image infarct area (r = 0.92 in the LAO view) appeared most promising for future clinical evaluation. These experimental relationships are analyzed and future patient application of these imaging techniques are considered.

There has been considerable recent interest in evaluating techniques for quantitatively assessing the size of acute myocardial infarction. Such techniques have included hemodynamic monitoring, precordial ST segment mapping,2 creatine phosphokinase (CPK) curve evaluation,3 and most recently, radioisotope infarct imaging.4-8 A variety of radiopharmaceuticals have been used to visualize regions of fresh myocardial infarction.4-8 Technetium 99m pyrophosphate (Tc-PYP), first applied to myocardial imaging by Bonte and co-workers,8 appears to be very useful for imaging fresh infarction.5 10-12 Only preliminary attempts, however, have been made to quantitate infarct size with this agent.10-11 Accordingly, we attempted to correlate infarct size, as estimated from the image recorded from the intact animal, with that estimated by pathologic analysis. In this process, agent distribution and its relation to tissue CPK content was carefully examined.

Methods

Fourteen mongrel dogs weighing 14 to 31 kg were anesthetized with intravenous sodium pentobarbital (5 mg/kg). Via a carotid approach, utilizing a modified coaxial catheter and a closed chest technique,13 we selectively embolized small occluded catheter segments to branches of the left coronary artery, creating discrete areas of myocardial infarction. The dogs were given an antiarrhythmic drug (lidocaine, 50 mg, intravenously preinfarction; 1 hour postinfarction, and as needed) and antibiotic (cephazolin, 500 mg, intravenously) prophylaxis at the time of the procedure, and allowed to recover. Standard 12 lead electrocardiograms were taken preinfarction and one and 48 hours postinfarction. Forty-eight hours following infarction the dogs were again anesthetized and injected intravenously with 15 mCi of Tc-PYP formulated in our laboratory.14 Seventy-five to ninety minutes later, infarct images were obtained in ten animals in the anterior, lateral, LAO and RAO projections. Two normal dogs were also imaged with Tc-PYP.

The dogs were sacrificed two hours following Tc-PYP injection, at which time the hearts were removed and the ventricles isolated. The right ventricle was separated from the interventricular septum anteriorly. The septum was freed from the posterior ventricular wall and the specimen laid open. A necropsy image was taken. In each experiment, using a cork bore biopsy tool, a total of 74 cylindrical, transmural specimens were taken from grossly normal, grossly infarcted, and marginal zones of both ventricles. These biopsy specimens were counted in a well counter, quick frozen in dry ice and alcohol, and then stored at

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-70°F until subsequently weighed and analyzed for CPK content according to the method of Rosalki. Similarly, biopsies contiguous to those analyzed for Tc-PYP radioactivity were histologically evaluated and graded on a scale of 0 to 2+. Grade 0 represented normal histology; 1+ showed edema, loss of cell margins, and striations or minimal white blood cell infiltration; and 2+ showed major white blood cell infiltration or frank necrosis. Gross areas of infarction were identified by careful dissection and weighed. Outline drawings of the individual ventricles, as well as the regions of gross infarction, were made and the respective areas were evaluated by planimetry.

All images were obtained using a scintillation camera (Pho Gamma III-Searle Radiographics, Inc.) equipped with a 4000 parallel hole, low energy collimator. The scintillation camera was interfaced to a small digital computer (Gamma 11—Digital Equipment Corporation) which produced images of the heart in a 64 × 64 array. Quantitation was performed by calibration with a radioactive grid of known dimensions. Measurements of Tc-PYP infarct area and infarct count rate obtained from both the intact animal in vivo and the opened heart specimen were compared with infarct area and weight determined from the gross specimen. Infarct images were clarified using routine digital contrast enhancement techniques available on the Gamma 11 data acquisition and analysis system. The entire gray scale of the image was bracketed between the maximum image infarct count rate and background count rate. Background activity was chosen from a region adjacent to the heart, utilizing a computer histogram, where activity was plotted as a function of position across the chest at the level of the infarct (fig. 1). The region of the infarct clarified by image enhancement was then outlined using a joystick. Areas in square centimeters and counts per infarct per minute were calculated for each infarct region in each view (fig. 2). Postmortem images were analyzed in a similar and equally objective fashion. Computer histograms, made to evaluate the distribution of counts across infarct regions, revealed a precipitous decrease in count rate over a narrow margin between infarcted and normal tissue. Based on these histograms and the position of infarcts in the gross specimens, it was determined that infarction was represented by all computer image locations with count rates greater than 25% of that image location having the maximum count rate. These infarct regions were then outlined using a joystick and areas in square centimeters and counts per minute per region of interest were calculated. Images with homogeneous activity were designated as not infarcted.

Finally, in each case, in vivo and postmortem in vivo and postmortem infarct area and activity were correlated with postmortem gross infarct area and weight. All images were taken to 300,000 counts and the time noted. All counts were normalized for decay and dose corrected for body weight.

Results
A total of 14 transmural infarctions were produced and all correlated well with the localization expected on the basis of plug placement and regional electrocardiogram changes. Twelve infarctions (ten anterior and two inferior), weighing 3.1 to 26.5 grams were analyzed. Ten were imaged in vivo, but the two smallest infarctions, weighing 3.1 and 5.1 grams respectively, and the hearts of the two normal dogs could only be seen in the postmortem preparation. Nine infarctions were imaged at postmortem examination. Images of nine infarcted animals were subjected to computer analysis both in vivo and at postmortem examination (fig. 3). Two infarcted animals were imaged only at the postmortem study.

There was a negative linear relationship between CPK content and Tc-PYP activity. When CPK was normalized for each experiment and plotted against Tc-PYP uptake, this negative correlation was strong (fig. 4). Pathologic grade was also clearly associated with enzyme content (fig. 5). Specimens with diffuse 2+ histology (dense infarction) showed high radioactivity and low enzyme content, while those with 1+ histology (spotty infarction) showed intermediate radioactivity and enzyme content, and those with Grade 0 histology (normal) were of low radioactivity and high enzyme content. All specimens found to contain infarcted tissue on histologic examination were also found to have reduced CPK content and increased Tc-PYP uptake.

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Postmortem infarct images of the opened heart correspond well with gross appearance (fig. 6). Computer estimated infarct area and gross infarct area correlated well by linear regression analysis \( (r = 0.95) \). Computer postmortem infarct image activity correlated well with gross infarct weight \( (r = 0.87) \).

There was good correlation between computer-estimated \textit{in vivo} infarct area and gross infarct area in the RAO and LAO projections (fig. 7). The lateral projection showed a poorer correlation \( (r = 0.68) \). The anterior projection could not be correlated, however, as bone radioactivity in this view often made delineation of infarction difficult without reference to other projections. \textit{In vivo} image radioactivity correlated with gross infarct weight, in the RAO and LAO projections (fig. 8) and less well in the left lateral projection \( (r = 0.75) \). There was a good correlation between computer-estimated \textit{in vivo} infarct area and gross infarct weight (fig. 9).

**Discussion**

In 1964, D'Agostino and coworkers first noted the deposition of electron dense material within mitochondria of necrotic myocardial cells.\textsuperscript{18} They later postulated that the material was a crystalline structure similar to hydroxyapatite of bone.\textsuperscript{17} Eight years later Shen and coworkers found greatly increased calcium deposition, 10 to 20 times normal, in myocardial cells.
Thus, calcium seemed to accumulate only in infarcted, but reperfused myocardium. One year later Bonte and coworkers demonstrated the deposition of Tc-PYP tracer in regions of fresh myocardial infarction in closed chest dogs. It is likely that the Tc-PYP might, in fact, bind to the electron dense infarcted after complete coronary occlusion. Increased calcium was noted only in areas reperfused following infarction. However, infarcted areas not reperfused showed normal intramyocardial calcium levels. Likewise, regions which were ischemic for less than 20 minutes showed normal calcium content.

Figure 4

Relationship between tissue CPK content and Tc-PYP uptake. There was a negative linear relationship between CPK content, normalized for each experiment and plotted on the horizontal axis, and Tc-PYP activity plotted on the vertical axis.

Figure 5

Relationship of histology to tissue CPK content and Tc-PYP uptake. Histologic grade, plotted on the horizontal axis, correlated closely with tissue CPK content (striped bars, left axis) and tissue Tc-PYP activity (solid bars, right axis).

Figure 6

Postmortem imaging. The location and size of the region of infarction diagrammed from the opened postmortem heart (A) and identified from the computer image of the same left ventricle (B) compared closely with that of the infarct (arrow) seen in the actual specimen above.)
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Gross infarct area versus in vivo infarct image area. There was a good correlation between computer estimated in vivo infarct image area and gross infarct area, best in the RAO view (left) and the LAO view (right).

Gross mitochondrial deposit by a mechanism similar to that by which it binds to bone. The exact mechanism is yet unknown.

Recently investigators have employed Tc-PYP and other radiopharmaceuticals in an attempt to quantitate the extent of myocardial infarction. Using Technetium 99m tetracycline, Holman and coworkers have shown a gross relationship in patients between peak serum CPK concentration and in vivo image infarct area. Using Tc-PYP, a good correlation was demonstrated between total serum CPK and maximal image infarct area in dogs. A similar correlation between infarct size estimated from peak serum CPK concentration and image infarct area has been recently reported using glucoheptinate. Willerson and coworkers have recently related image infarct size and gross infarct weight. Such quantitation of infarction could be of critical importance in determining patient prognosis and in guiding therapy.

It has been shown that CPK depletion is proportional to the weight of infarcted tissue. In this paper, we have demonstrated a negative correlation between CPK content and Tc-PYP accumulation. Further, we could reasonably predict the area and weight of infarction in the heart at postmortem examination. Most important, it was also possible to predict the area and weight of infarction in the living animal. In fact, knowing the image infarct area, we could quite accurately estimate the gross infarct weight, likely the most critical factor in prognosis of patients with infarcts. This area-weight relationship would not be unexpected with a fairly homogeneous thickness of the involved left ventricular wall. Variations in the thickness, or involvement of papillary muscles, could radically alter this relationship, however.

Our results could be more easily analyzed because the majority of the infarctions studied were anterior rather than inferior and nearly always involved the apex. Thus, only rarely did the damaged area present a flat configuration in a profile view and a much larger region when looking at the infarct face on. The specimen which fit our in vivo relationship most poorly demonstrated prominent septal involvement. The infarct was thus seen in profile in the LAO view and underestimated compared to its image in other projections. In our experiment the best correlation of gross infarct area with in vivo image area (in the LAO view) was equaled when evaluated by the method of Harris and coworkers (table 1).

It was possible to predict the area and weight of infarction when projected on a flat configuration, whether a profile view or LAO view. These results could be more easily analyzed because the majority of the infarctions studied were anterior rather than inferior and nearly always involved the apex.

Infarct weight versus in vivo infarct image area. In vivo infarct image area in the LAO view (left) correlated well with infarct weight. The correlation was equally good using the view projecting the largest measured area (right).
Table 1

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<tr>
<th>Gross infarct area</th>
<th>Infarct weight</th>
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<tr>
<td>Postmortem Computer Estimated</td>
<td>Infarct Area 0.95</td>
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<tr>
<td>In vivo Computer Estimated</td>
<td>Infarct Area 0.79 — RAO</td>
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<td>Infarct Area 0.72 — LAO</td>
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<td>Infarct Area 0.68 — Left Lateral</td>
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<td>Infarct Activity 0.75 — Left Lateral</td>
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Presented here are the results of all significant correlations among all computer image estimated parameters (infarct area and activity), and those measured from the gross specimen (infarct area and weight). The numbers represent r values.

the ability and necessity to enhance the images with computer techniques.

We found no biopsy specimen revealing evidence of infarction which did not reveal an elevated pyrophosphate content. Other authors have described "doughnut" shaped in vivo infarction images.11 Such regions have occasionally been shown to have centrally infarcted zones lacking white blood cell infiltration.11 It is postulated, as in Shen’s model,18 that the total exclusion of blood supply does not permit Tc-PYP access to this central zone. However, when we have seen this "doughnut" in vivo, postmortem analysis failed to reveal any central necrotic zone with low Tc-PYP content. In fact, our infarctions were usually grossly hemorrhagic and always with leukocyte infiltration which seemed generally proportional to infarct density judged by tissue CPK content.

In our study, Tc-PYP accumulation was proportional to myocardial CPK depletion. It is likely, however, that under certain conditions factors other than infarct density would influence myocardial pyrophosphate uptake and alter this relationship. Coronary blood flow is likely one of these factors and the effects of variations in coronary blood flow on Tc-PYP uptake will be of great importance in resolving this problem. When they created infarcts by coronary occlusion in rabbits, Kjehshus and Sobel found a direct relationship between relative coronary blood flow and myocardial CPK content.21 The most dense infarct zone, therefore, would have the lowest relative blood flow. In our study, Tc-PYP tissue labeling was active in this zone. Possibly, relative flow in our model 48 hours postinfarction, even at its lowest level, was great enough to permit cellular Tc-PYP accumulation. This may be due to the nature of our model or the timing of our experiment. Apparently, the "doughnut" phenomenon only occurs in association with large infarcts and proximal left coronary obstructions.11 Such infarcts were not present in our animals, and in fact, were avoided in an effort to promote animal survival. Also, infarct visualization may become more uniform with the passage of time and the increasing perfusion brought by collateral flow or the release of spasm. Of course, the relationship of coronary flow to Tc-PYP localization and accumulation must be investigated and will be a crucial factor in determining our ability to quantitate infarction in man by imaging methods.

Correlation of image infarct radioactivity with infarct weight was good here, but likely will be more difficult to determine in patients. Our animals were nearly all of similar weight with minimal and likely uniform chest thickness. Further, factors such as exact dose and time of imaging were rigidly controlled. While the former may be controlled, tracer accumulation has been shown to vary with infarct age19 and may make the index of image activity difficult to apply to the ill infarct patient. Infarct image area, however, may be an accurate index of infarct size, preserved to the limits of visualization and must be investigated. Additionally, attenuation factors will likely make this relationship more difficult to apply in the clinical setting.23 Attenuation of Tc-PYP activity will depend on patient size and infarct location. This factor will likely compromise the relationship between infarct size and Tc-PYP activity. The effect of this attenuation on the estimation of infarct size from image infarct area remains to be determined. Again, it would appear that the relationship between in vivo image infarct area and gross infarct weight (fig. 9) represents the most valuable possibility for infarct quantitation in living man using infarct labeling techniques.

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