A Comparison of Infarct Identification with Technetium-99m Pyrophosphate and Staining with Triphenyl Tetrazolium Chloride

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The topographic relationship between the uptake of technetium-99m pyrophosphate (PPi) and myocardial infarction delineated by 2,3,5-triphenyl tetrazolium chloride (TTC) was studied in a canine model of permanent coronary occlusion (24–48 hr). Photographs of TTC staining and scintigraphic images of PPi uptake were planimetered for infarct area. In addition, narrow tissue samples (3 × 10 mm) were taken on both sides of the TTC border and counted for PPi uptake. A significant correlation ($p < 0.001$) was found between area of PPi uptake and area of myocardium unstained by TTC ($r = 0.84$ in epicardium and $r = 0.91$ in endocardium). The slope relating PPi to TTC for all infarcts was 1.01 ± 0.11, indicating that variations in infarct size were followed equally by the two techniques. Tissue counting showed the ratio of PPi activity just inside the infarct to activity just outside the infarct to be 9.2 ± 0.6 (mean ± s.e.m.). Thus, PPi is distributed topographically in a manner identical to the distribution of irreversibly injured myocardium as delineated by TTC.

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The location and extent of damaged muscle occurring in acute myocardial infarction are important determinants of patient prognosis (*1–5*). Therefore, several methods have been developed to locate and size acute myocardial infarcts. Principal among these are precordial electrocardiographic mapping (*6,7*), measurements of the release of creatine kinase (CK) and the CK-MB and CK-B isoenzymes (*8–11*), and myocardial scintigraphy with the infarct-avid agent technetium-99m pyrophosphate (PPi) (*12–19*). Although maximal PPi uptake generally does not occur until 1–3 days following infarction, scintigraphic techniques are particularly valuable because they provide a visual assessment of both infarct location and size.

Scintigraphic imaging of myocardial infarction with PPi is based upon the assumption that PPi uptake is confined primarily to irreversibly damaged muscle cells. Previous animal studies from our laboratory (*15,16,20,21*) and elsewhere (*14,22–24*) support that assumption, but some clinical studies have suggested that PPi uptake may occur in reversibly injured tissue (*25–27*). The dehydrogenase stain 2,3,5-triphenyl tetrazolium chloride (TTC) is reduced by active dehydrogenase enzymes to a brick-red color in normal myocardium (*28–31*). In irreversibly injured myocardium, dehydrogenase enzyme activity is depleted, and thus TTC remains unreduced. Infarcted tissue is thus identified as a palely colored area surrounded by normal, stained myocardium. In this study, we tested the hypothesis that PPi uptake is restricted on a macroscopic scale to irreversibly injured myocardium following experimental coronary artery occlusion, by directly correlating PPi distribution with the area of myocardium unstained by TTC.

METHODS

Seventeen adult mongrel dogs of either sex were anesthetized with 30 mg/kg sodium pentobarbital and ventilated with room air on a Harvard respirator. Under sterile conditions a left thoracotomy was performed through the fifth intercostal space, and the heart was

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isolated in a pericardial cradle. The left anterior descending coronary artery (LAD) was permanently occluded just distal to its first diagonal branch in 13 dogs, and the circumflex coronary artery (CX) was occluded just distal to its first descending branch in four dogs. The pericardium was loosely approximated and the thoracotomy closed. The dogs were allowed to recover for a period of 24–48 hr.

Following recovery, 10–15 mCi (0.3 mCi/kg) of PPi was administered intravenously, and three hours later the animal was killed by barbituate overdose (*19*). Hearts were excised and all fat, both atria, and the right-ventricular free wall were removed. The left ventricle was cut from base to apex at the posterior junction of the septum and free wall, and unfolded to form a slab. The slab was sliced longitudinally into separate epicardial and endocardial halves. Each was immersed in a 1% TTC solution until normal myocardium was stained brick red (*30*), then washed with normal saline so that unstained (infarcted) myocardium appeared pale. Both faces of each slab were photographed adjacent to a metric ruler.

Following photography, scintigrams of each slab were obtained with a 37-photomultiplier tube, mobile gamma camera.* The slabs were placed flat on the surface of a high-resolution parallel-hole collimator. At least 300,000 counts were acquired in each image. Images were obtained in the presence of point sources set 5 cm apart for subsequent scaling. The slab perimeter was outlined with a point source. Data were acquired in a 128 × 128 digital matrix and stored in a video image processor.† Subsequently, images were displayed and photographed.

In three dogs with LAD occlusions, a section 1 cm wide was cut simultaneously from the center of both epi- and endocardial slabs so as to include normally stained septum, unstained infarct, and normally stained free wall. Samples of unstained and stained myocardium were obtained along both septal and free-wall borders. Sequential samples 2–3 mm wide were taken for approximately 6 mm on either side of the carefully dissected TTC border. Samples were counted for Tc-99m activity in a NaI well counter using a multichannel analyzer.

In the remaining 14 dogs, images of either TTC-stained slabs or PPi images were enlarged to life size. Both slab perimeters and infarct perimeters were outlined independently by two experienced observers. In those infarcts producing a "doughnut" scintigram (*20*), the outermost perimeter of PPi uptake was taken to delineate the infarcted area. This resulted in up to six tracings per dog: four TTC tracings (each side of each slab) and two PPi tracings (epicardium and endocardium). In some dogs the tough fibrous epicardial and endocardial surfaces hindered uniform TTC penetration, preventing accurate infarct delineation. However, cut surfaces always demonstrated clear staining. Therefore, only tracings of TTC areas on cut surfaces were used in this study. Each tracing was planimetered with a Talos digitizing pad. Total LV surface area and total infarct surface area (both cm^2) were measured for each slab.

Linear regression analysis was used to compare TTC and PPi measurements of infarct size. Pearson correlation coefficients were computed, and a p value of less than 0.05 was required for statistical significance.

RESULTS

Typical results obtained with TTC staining and PPi imaging of LV slabs are shown in Figs. 1, 2, and 3. These three figures represent varying degrees of PPi uptake in infarcted myocardium in three separate dogs. In Fig. 1, PPi uptake is seen throughout the entire area unstained by TTC, most likely because sufficient blood flow remained throughout the infarcted area to permit PPi delivery. In Fig. 2, PPi uptake is seen throughout all but the most central regions of the infarct, where blood flow was probably reduced so severely that little PPi could be delivered. Finally, in Fig. 3, a typical example of a large "doughnut" PPi scintigram and its corresponding TTC photograph are shown. Note that PPi uptake occurred primarily along the perimeter of the infarct. However, the decreased activity in the interior of the PPi scintigram was composed entirely of unstained, and therefore irreversibly injured, myocardial tissue. In all three types of PPi scintigrams the topographical distributions of infarcted myocardium indicated by the two techniques were similar, provided that the interiors of "doughnut"

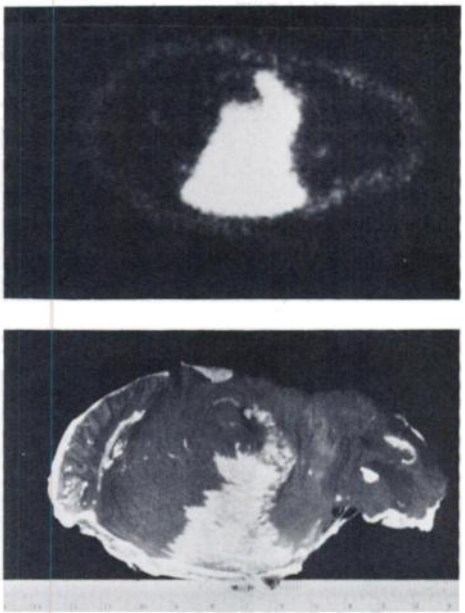

FIG. 1. Comparison of Tc-99m pyrophosphate (PPi) uptake with myocardium unstained by triphenyl tetrazolium chloride (TTC) in excised canine left ventricle. LV was cut from base to apex at posterior junction of septum and free wall to form a slab. TTC-unstained myocardium represents irreversibly injured cells. PPi uptake is noted throughout entire area unstained by TTC.

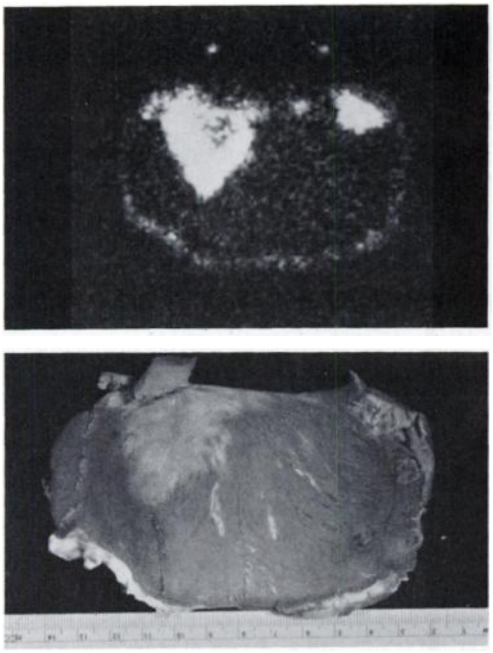

FIG. 2. Comparison of PPi uptake with myocardium unstained by TTC in a mild "doughnut" infarct. LV preparation is as described in Fig. 1. Note small central region of decreased PPi uptake, probably corresponding to extremely reduced blood flow. Interior region of PPi "doughnut" is entirely unstained by TTC.

scintigrams were considered irreversibly injured. In this study five "doughnut" infarcts were created, and in every case of decreased activity the region was composed entirely of irreversibly injured myocardium.

The correlations between the two techniques for quantifying infarct size are shown in Figs. 4 and 5. Total LV areas for the two techniques were compared to demonstrate that PPi and TTC images were enlarged to the same scale. There was a good correlation between the total left-ventricular areas assessed by the two techniques ($r = 0.87$ subepicardium, $r = 0.95$ subendocardium). The positive intercepts indicate a slight overestimate of true ventricular areas by PPi scintigrams. Determinations of LV area and infarct area were consistent between the two observers. The interobserver coefficient of variability was 3.1% for TTC and 2.8% for PPi.

There was a strong correlation between infarct areas using these two techniques. In both subepicardium and subendocardium, the slopes of the regression equations were not significantly different from unity (Fig. 5). Also, in both cases the intercepts were positive, indicating that PPi slightly overestimated infarct size. This slight overestimation of the distribution of any increased activity marker is expected because of the limited resolution of gamma-camera collimator imaging systems. If the subepicardial and subendocardial data are combined, the slight but consistent overestimation of areas by PPi was 6.9 ± 2.2 cm^2 (mean ± s.d.).

The slope relating PPi to TTC unstained area for all infarcts was 1.01 ± 0.11 (±s.d.), indicating that changes in infarct size were followed equally by both techniques. In the subepicardium, the correlation between PPi and TTC infarct size was $r = 0.84$, while in the subendocardium it was $r = 0.91$. In both cases the correlations were statistically significant ($p < 0.001$).

Tissue-counting results are summarized in Fig. 6, where relative PPi activity is plotted as a function of distance from the TTC infarct border. Epicardial, endocardial, and total left-ventricular samples all demonstrate a sharp difference in Tc-99m activity at the TTC infarct border. The ratio of PPi activity in the first sample inside the infarct to that in the first sample outside the infarct was 9.2 ± 0.6 (mean ± s.e.m.).

DISCUSSION

The purpose of this study was to determine whether Tc-99m pyrophosphate uptake in the myocardium following coronary artery ligation is restricted on a macroscopic scale to areas of irreversibly damaged (necrotic) myocardium. Previous experimental studies suggesting such a relationship have not evaluated a direct topographical correlation between total infarct area and area of PPi uptake. In the current study, infarction was delineated by using the dehydrogenase stain 2,3,5-triphenyl tetrazolium chloride. Superimposition of photographic

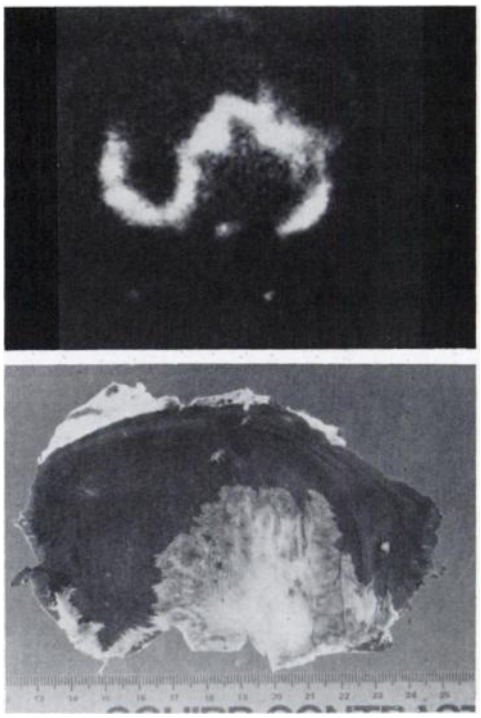

FIG. 3. Comparison of PPi uptake with myocardium unstained by TTC in a large "doughnut" infarct. LV preparation is as described in Fig. 1. In this case it is likely that blood flow was so severely reduced that PPi was delivered only to perimeter of infarct. Note, however, that PPi perimeter entirely surrounds the myocardium unstained by TTC.

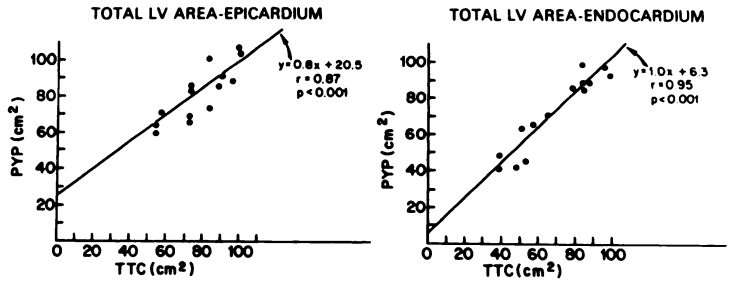

FIG. 4. Linear regression analysis comparing scintigraphic and in vitro photographic planimetry of total ventricular areas. Pearson r values are indicated for each correlation (N = 14).

(TTC) and scintigraphic (PPi) images permitted direct correlation of infarct area. Tissue-counting of PPi uptake in normal and infarcted myocardium could be guided by TTC staining. The results of this study clearly indicate a strong correlation between the distribution of PPi uptake and the distribution of myocardium unstained by TTC.

Our results are in agreement with several previous studies. Buja et al. studied the correlation of PPi uptake with mitochondrial calcification, and found elevated calcium levels at all sites of increased PPi uptake (*21*). Similar results were reported by Reimer et al. (*23*) for ischemic cardiac muscle, and by Siegel et al. (*32*) for ischemic skeletal muscle. Buja et al. also reported autoradiographic evidence of Tc-99m PPi uptake in cells with histologic features of advanced necrosis and in a small population of damaged border-zone cells with lipid droplets and focal, early mitochondrial calcification (*21*). No PPi uptake was seen in areas without severely injured muscle cells. Marcus et al. (*24*) compared PPi uptake with classical histological criteria for myocardial infarction, including fiber fragmentation, karyolysis, and polymorphonuclear cell infiltration, and found no PPi uptake in tissue areas without necrosis. Dewanjee and Kahn (*33*) compared the cellular localization of PPi with Ca-45 uptake and found a strong correlation between PPi uptake, increased calcium deposition, and infarcted tissue. Botvinick et al. (*14*) examined myocardium in which blood flow was only slightly reduced following coronary occlusion, and found significant PPi uptake only in necrotic or severely injured cells. In areas of reduced perfusion without PPi uptake, no necrotic or severely injured myocardium was identified.

However, questions have still arisen concerning PPi uptake in ischemic but reversibly injured myocardium. Our previous and present findings suggest that PPi uptake occurs only in irreversibly injured myocardium. Reimer et al. (*23*) found no permanent myocardial injury and no PPi uptake in animals subjected to 15 min of total coronary occlusion followed by reperfusion. However, PPi uptake and massive tissue calcification were observed in six of 10 dogs following 40 min of occlusion. Coleman et al. (*22*) performed a similar comparison of PPi uptake with increased CK-MB serum activity. With less than 20 min of occlusion, neither increased CK-MB nor increased PPi uptake was observed. With more than 30 min of occlusion, however, both elevated CK-MB activity and increased PPi uptake occurred. Thus, in these previous studies, ischemia without infarction was not associated with increased PPi uptake.

A "doughnut" pattern of PPi uptake may be observed in animals and patients with proximal LAD occlusion or severe narrowing (*16,21,34,35*). Since PPi delivery is dependent upon residual myocardial blood flow, low-flow regions near the center of large infarcts may fail to take up PPi (*16,21,34,35*). Several dogs in this study demonstrated "doughnut" scintigrams, and in each case the area surrounded by intense PPi uptake was infarcted. This characteristic pattern is caused by failure of tracer to reach the necrotic tissue at the center of the infarct (*16,21,35*).

In this study, we have shown that the spatial distribution of PPi is equivalent to the spatial distribution of myocardium unstained by TTC. PPi uptake was restricted predominantly to infarcted myocardium. The slight overestimation of infarct size with PPi relative to TTC is expected and is explained by the relative spatial resolutions of the techniques. This information, in combination with previous studies of in vivo sizing, in-

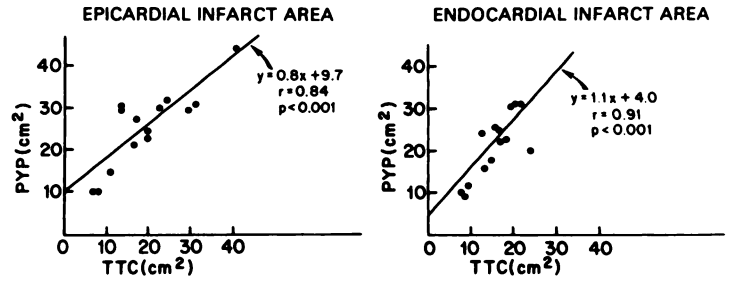

FIG. 5. Linear regression analysis of ventricular infarct areas correlating PPi uptake and TTC-unstained myocardium. Pearson r values are indicated for each correlation (N = 14).

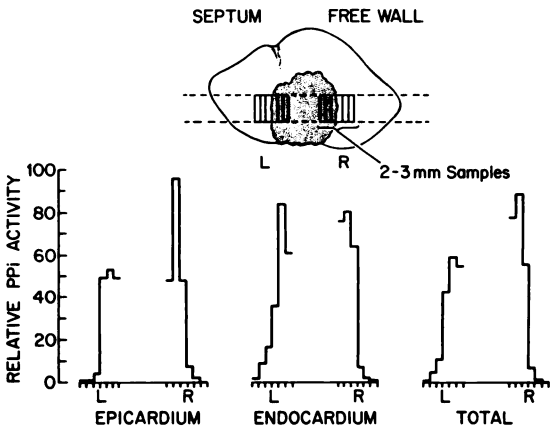

FIG. 6. Relative PPi uptake as a function of distance from TTC border. Upper part of figure shows schema of tissue-sampling procedure. Data represent average of three dogs.

dicates that macroscopically observed PPi uptake is confined to irreversibly injured myocardium.

FOOTNOTES

* Technicare 420.
† Technicare 550.

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