INVESTIGATIVE NUCLEAR MEDICINE

Technetium-99m(Sn^{2+})Pyrophosphate in Ischemic and Infarcted Dog Myocardium in Early Stages of Acute Coronary Occlusion: Histochemical and Tissue-Counting Comparisons

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We have investigated the pattern of accumulation of Tc-99m(Sn^{2+})pyrophosphate (Tc-99m PPi) in myocardial tissue of dogs during the early stages of acute occlusion of the left anterior descending coronary artery. Three groups were studied after: (a) 40 min occlusion followed by 6 hr reperfusion (n = 6); (b) 6 hr occlusion followed by one hour reperfusion (n = 5); and (c) 7 hr occlusion with no reperfusion (n = 4). Areas of myocardial infarction were defined with triphenyl-tetrazolium chloride (TTC) staining, and blood flow was determined with 9-μ radioactive microspheres. In Group C uptake in infarcted and peri-infarct areas was not enhanced, most likely owing to low flow. In Group B, with late reperfusion, Tc-99m PPi sequestration was increased in both infarcted and peri-infarcted tissues. In Group A, areas ischemic during occlusion but with normal flow and viability by TTC after 6 hr of reperfusion showed significant uptake of Tc-99m PPi (twice the uptake of nonischemic regions).

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At the present time, controversy exists regarding the extent to which Tc-99m(Sn^{2+})pyrophosphate (Tc-99m PPi) is accumulated in ischemic noninfarcted myocardial tissue. One experimental study performed 48 hr after coronary occlusion showed a small (1–5 mm) peri-infarcted zone of increased Tc-99m PPi uptake (*1*), whereas another investigation using Tc-99m glucoheptonate reported a similar peri-infarcted area of increased radionuclide uptake 3–8 hr after inception of acute coronary occlusion (*2*).

A most useful model of acute myocardial ischemia and infarction has been proposed by Reimer and Jennings (*3*). They described the course of infarction in terms of a wavefront of necrosis that begins in the innermost endocardium after about 40 min of occlusion. Over the next 3–6 hr, the remaining mid- and epicardial layers succumb so that by approximately 6 hr, acute experimental infarcts are, by and large, transmural. The "area at risk" of necrosis has largely been infarcted, and because of the nature of the coronary microvasculature as end-arterioles, little ischemic lateral border zone can be detected (*3*). Although the model proposed by Reimer and Jennings strictly pertains to circumflex coronary occlusions—which allow less collateral blood flow to the posterior papillary muscle and result in more homogeneous ischemia—other investigators (*4*) have used it to describe distribution of transmural necrosis after ligation of the left anterior descending coronary artery (LAD) in the dog.

No opportunity has existed in previous investigations (*5,6*) for assessing Tc-99m PPi sequestration in ischemic but not infarcted myocardium, since no well-defined

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ischemic tissue can be demonstrated histologically at 24 hr after coronary occlusion (*7*). The present investigation was therefore designed to evaluate the accumulation of Tc-99m PPi in ischemic and necrotic myocardium early in the course of acute coronary occlusion, by utilizing three experimental conditions: (a) 40-min occlusion with 6-hr of reperfusion; (b) 6-hr occlusion with 1 hr of reperfusion; and (c) 7-hr occlusion without reperfusion. Tc-99m PPi was injected shortly after release of occlusion (a and b) or 1 hr before sacrifice (c), and its tissue concentration was measured by tissue counting, whereas areas of necrosis were identified histochemically using triphenyl-tetrazolium chloride (TTC); regional blood flow was determined with 9-μ radioactive microspheres.

METHODS

Experimental animals. Twenty dogs of either sex, (14–22 kg) were anesthetized with 450–650 mg of sodium pentobarbital i.v. The trachea was intubated and respiration maintained by a Harvard respiratory pump. Blood gases and pH were measured and kept within physiological ranges by appropriate ventilation. The femoral artery was cannulated for measurement of arterial pressure, and the vein for fluid administration. A left lateral thoracotomy was performed and the exposed heart was suspended in a pericardial cradle. The left atrium was cannulated for administration of radioactive microspheres. Using sharp dissection, the LAD was isolated below its first diagonal branch. LAD occlusion was done with a vascular clamp. Five dogs were excluded from the study because of irreversible ventricular fibrillation.

Duration of LAD occlusions. The experimental design was tailored to fit the Reimer and Jennings model of acute myocardial infarction (*3*). This model was derived from experiments consisting of acute ligation of the circumflex coronary artery, so our experimental design was not identical to that of Reimer and Jennings. However, ours provided a pathophysiologic framework for the study of Tc-99m PPi accumulation in ischemic and in peri-infarcted myocardial regions. Accordingly, three experimental groups were selected:

1. Group A: Forty-minute LAD occlusions, 6-hr reperfusion. To study myocardial sequestration of Tc-99m PPi in a major territory of the LAD area-at-risk that had not yet undergone cardiac necrosis, LAD occlusions in 6 dogs were maintained for 40 min. After this interval, reperfusion was provided and maintained for 6 hr. Tc-99m PPi (17 to 22 mCi) was given i.v. shortly after the start of reperfusion. In all the animals of this group, relative myocardial blood flows were investigated using tracer microspheres 9 ± 2 μ (s.d.) in diameter. They were administered into the left atrium after 10 to 15 min of firm agitation. The microsphere species used were tagged with Sn-113, Cr-51, and Sc-46. Four million spheres of each species were given for the measurement of flow, which was determined before and during LAD occlusion and at the end of the reperfusion period. The dogs were killed by intracardiac administration of KCl (14 mEq).

2. Group B: Six-hour LAD occlusion, 1-hr reperfusion. In five dogs, LAD occlusion was maintained for 6 hr. The aim was to study Tc-99m PPi uptake in established infarcted and peri-infarcted tissue, and to compare the data with investigations that looked at Tc-99m PPi myocardial accumulation later in the course of myocardial infarction (*1*,*6*,*8*,*9*). To ensure delivery of the radiopharmaceutical, the occlusion was released at the end of the sixth hour and reperfusion allowed for 1 hr. Tc-99m PPi (16–30 mCi i.v.), was given immediately upon release of LAD occlusion (as in Group A animals). The wide range of Tc-99m PPi injections to dogs of this group was unintended, and probably had no effects on results, since in the investigation of Marcus et al. (*9*) the Tc-99m PPi dose range was 2–15 mCi, a sevenfold difference. No microsphere flow determinations were carried out in this group because data on regional blood flows, Tc-99m PPi localization, and histology in established infarcted and peri-infarcted tissues have been reported (*1*,*8*,*9*). Sacrifice was also with intracardiac KCl solution.

3. Group C: Seven-hour LAD occlusion, no reperfusion. In four dogs, LAD occlusion was maintained for 7 hr. To assess the effect of the flow-limited delivery of Tc-99m PPi to infarcted and peri-infarcted areas in animals with acute occlusions, the radiotracer was given (25–38 mCi i.v.) at the end of the sixth hour of occlusion. The animals were kept alive for another hour, at the end of which they were killed as in Group A. The last animal of Group C had microsphere flow determinations as described for Group A animals.

Histochemical identification of infarct areas. Immediately after sacrifice, the heart was immersed in chilled normal saline solution. The left ventricle (LV) was dissected free. Transverse LV slices (0.5–1.0 cm) were cut with a sharp blade, starting at the LV apex and continuing up to the level of the LAD ligature site. The slices were then incubated in triphenyl-tetrazolium chloride (TTC) at 37° for 20 min (*10*). TTC staining is a histochemical method for *macroscopic* identification of areas of acute cardiac necrosis. The spatial resolution of TTC staining is close to that of human vision, i.e., ~0.5 mm. On TTC staining, viable dehydrogenase-containing cardiac muscle appears brick red; necrotic myocardium, depleted of dehydrogenase, is pale (Fig. 1). The ability of this method to identify 3 to 6-hr acute cardiac necrosis has been established by histologic and electron-microscopic criteria (*11*). In each slice, the necrotic and nonnecrotic tissues were readily identified by immersing the TTC-incubated sections in buffered (10%) formalde-

FIG. 1. Typical appearance of noninfarcted (dark) and infarcted (pale) myocardium in a Group II dog with transmural infarction. Note sharp demarcation between contrasting areas.

hyde. This process enhanced differences between brick red and pale colors in the sections.

Tissue sampling and in vitro counting. In two Group A animals, myocardial sampling (6–8 samples of 0.5–1.0 g/dog) was guided by TTC staining, or lack thereof, in the endocardial and epicardial halves of the LAD-dependent territory. Control samples were those located opposite the ischemic zone (i.e., nonischemic territory). In the remaining four Group A animals, in which myocardial sections showed homogenous TTC staining at autopsy, 2–10 samples (0.5–1.0 g) per dog were taken from the endocardium and epicardium of the area subserved by the LAD, and from the remote nonischemic zone.

In all the animals of Groups B and C, 2–6 samples (0.5–1 g) per dog were selected from the center of the TTC unstained area of cardiac necrosis and from a TTC-stained myocardial area located 5–10 mm away from the necrotic area (the "peri-infarction area," *1,3,12*). Sampling of nonischemic myocardium was as in Group A. Samples were assayed for Tc-99m PPi content and for radionuclide microsphere activity using a gamma counter. All values are expressed as the ratio of cpm/g heart in the tissue of interest to the cpm/g myocardium in the nonischemic tissue of the same transverse slice. In all animals in Groups B and C, and in the two animals in Group A that developed myocardial

TABLE 1. TISSUE-COUNTING DATA

A. 40-min LAD oclusions, 6-hr reperfusion (6 dogs)

	Microsphere flow during occlusion (% nonischemic flow)	Microsphere flow during late reperfusion (% nonischemic flow)	Tc-99m PPi uptake (relative to nonischemic tissue)
Subgroup I TTC-stained nonischemic, noninfarcted myocardial slices (n = 6)	0.94 ± sd 0.09‡	0.99 ± 0.25	1.16 ± 0.30
	NS		p <0.001
Subgroup II TTC-stained, ischemic but noninfarcted myocardial slices* (n = 20)	0.49 ± 0.34	0.82 ± 0.22	2.10 ± 0.29
	p <0.01		
Subgroup III Infarcted regions, TTC-nonstained (n = 7)	0.14 ± 0.06	0.39 ± 0.21	14.07 ± 10.3
		p <0.05	p <0.02
Peri-infarction regions, TTC-stained (n = 7)	0.15 ± 0.05	1.30 ± 0.97	2.12 ± 1.46
	(NS)†		

* LAD-dependent territory, viable by TTC but with depressed flow during occlusion.

† Microsphere flow during ischemia: infarcted vs. peri-infarcted regions not significantly different.

‡ All values as means ± standard deviation of the mean. Statistical differences assessed by Student's t-test. Each subgroup consisted of 2 dogs.

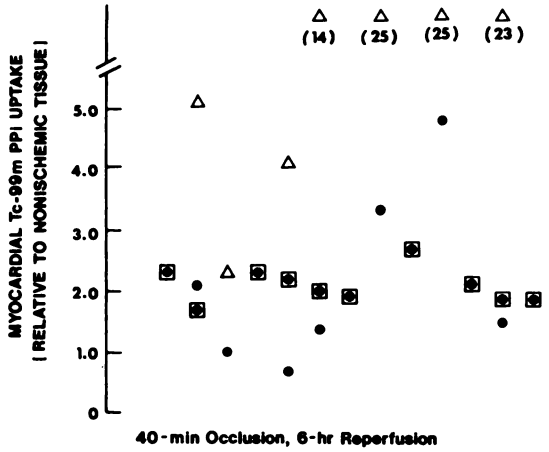

FIG. 2. Tc-99m PPi uptake (relative to nonischemic uptake) after 40-min LAD occlusions and 6-hr reperfusion. Symbols: (⊡) = TTC-stained regions that were ischemic but not infarcted; (●) = TTC-stained peri-infarcted regions; (△) = TTC-unstained infarcted regions.

infarction, areas were matched for location. They were designated as infarcted, peri-infarcted, and normal for Group B and C animals. They were designated subendocardial, subepicardial, and normal for Group A animals. In the four Group A animals that had normal TTC staining, samples from the LAD-dependent territory were pooled and compared with samples from the nonoccluded territory.

We considered that microsphere loss from the infarcted cardiac muscle was not a significant problem in the flow measurements in Group A, since Murdock and Cobb (*13*) have not been able to demonstrate shedding of 9-μ spheres at 6 hr after acute myocardial infarction.

Statistics. All values are expressed as mean ± standard deviation of the mean. Statistical differences among variates were assessed using Student's t-tests (paired and unpaired).

RESULTS

Table 1 shows the relationships between myocardial TTC staining, regional coronary flow, and Tc-99m PPi uptake in the Group A dogs (40-min LAD occlusion and 6-hr reperfusion). Dogs were subgrouped as follows: (I) showing normal TTC staining throughout and normal flow during occlusion (two dogs, six pooled nonischemic slices); (II) normal TTC staining but depressed flow during occlusion (two dogs, 20 pooled ischemic slices). Reperfusion flows in all these samples were 55% (or more) of the flow in the nonischemic area, and therefore indicated tissue viability (*14*). Subgroup III showed no TTC staining in the endocardial half of the area at risk, i.e., infarction with depressed reperfusion flow (seven slices). The adjacent epicardial zones had normal TTC staining, depressed flow during ischemia, and normal reperfusion flows (seven slices). These epicardial layers were designated as the 'peri-infarction region.' Thus, subgroup III consisted of the two dogs that developed subendocardial infarction.

In the six myocardial slices of the two dogs that had neither myocardial ischemia nor myocardial necrosis (subgroup I), myocardial blood flow was 0.94 ± 0.09 (relative to the nonischemic area) during occlusion, and was 0.99 ± 0.25 during late reperfusion (NS). TTC staining was brick red. As expected, there was no enhanced accumulation of Tc-99m PPi. However, in the 20 myocardial slices in subgroup II, the flow during occlusion was 0.49 ± 0.34, increasing significantly to 0.82 ± 0.22 during reperfusion ($p < 0.001$). These slices were

TABLE 2. TISSUE-COUNTING DATA

B. 6-hr LAD occlusion, 1-hr reperfusion (5 dogs)

	Tc-99m PPi uptake (relative to nonischemic tissue)
Infarcted, TTC-unstained tissue (n = 11 myocardial slices)	8.19 ± 4.49
	NS
Peri-infarcted, TTC-stained tissue (n = 10 myocardial slices)	4.17 ± 3.33

C. 7-hr LAD occlusion, no reperfusion (4 dogs)

	Tc-99m PPi uptake (relative to nonischemic tissue)
Infarcted, TTC-unstained tissue (n = 8 myocardial slices)	0.60 ± 0.35
	NS
Peri-infarcted, TTC-stained tissue (n = 8 myocardial slices)	1.01 ± 0.62

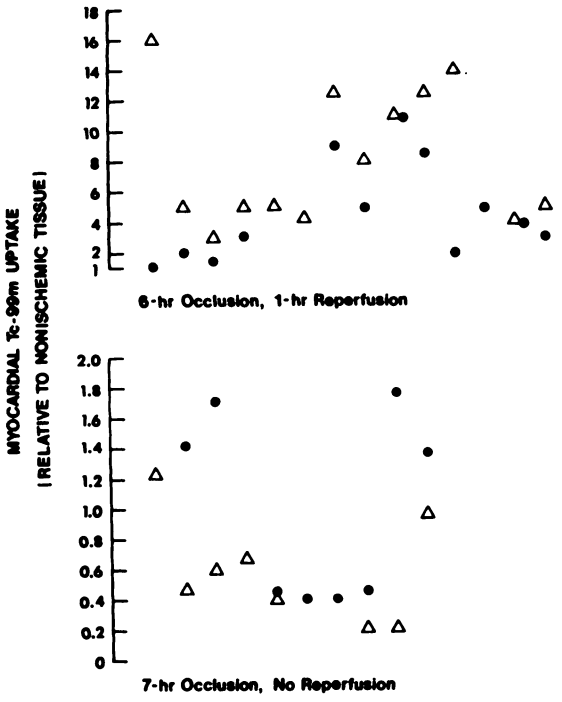

FIG. 3. Tc-99m PPi uptake relative to nonischemic uptake. Upper panel = 6-hr LAD occlusion, 1-hr reperfusion. Lower panel = 7-hr LAD occlusion, no reperfusion. Symbols as in Fig. 2.

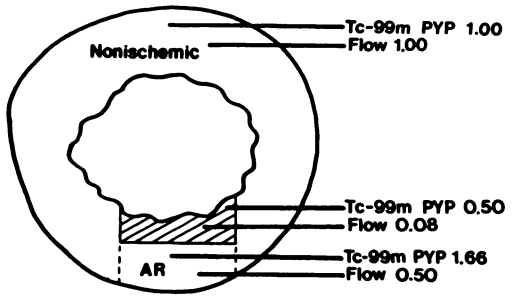

FIG. 4. Tc-99m PPi uptake and microsphere coronary flow in transverse section from dog with 7-hr LAD occlusion without reperfusion. Three areas are shown: (a) nonischemic region, (b) infarcted (by TTC) endocardial layer (shaded region), and (c) epicardial area at risk (AR). The epicardial area at risk showed maximal Tc-99m PPi uptake, whereas it had only 50% of nonischemic flow.

viable by TTC at 6 hr after reperfusion. (Whether a TTC-stained myocardial sample excludes presence of scattered groups of nonviable cells will be considered in the discussion.) Significantly, these slices showed increased Tc-99m PPi uptake (mean 2.10 ± 0.29 compared with nonischemic areas, $p < 0.001$; circles contained in squares in Fig. 2). Thus, seemingly viable cell populations as judged by reperfusion flow (*14*) exhibited abnormal radiophosphate uptake. In subgroup III (two dogs), seven myocardial slices from the endocardial half did not take the TTC stain and had low occlusion and reperfusion flows, i.e., 0.14 ± 0.06 and 0.39 ± 0.21. They showed increased Tc-99m PPi uptake and were considered to have undergone cardiac necrosis. The remaining seven epicardial slices had occlusion flows of 0.15 ± 0.05, which recovered after reperfusion to 1.30 ± 0.97. These slices took up TTC stain but nevertheless had abnormal accumulation of Tc-99m PPi.

Table 2 shows tissue-counting data for the nine animals of Groups B and C. In five dogs, Tc-99m PPi was given 6 hr after LAD occlusion, followed by 1 hr reperfusion. Both TTC-infarcted and peri-infarcted regions exhibited increased Tc-99m PPi accumulation equally (upper panel, Fig. 3). On the other hand, in the four dogs undergoing 7-hr LAD occlusion without reperfusion, only a few myocardial sections showed evidence of Tc-99m PPi sequestration (lower panel, Fig. 3). Statistically, neither the infarcted nor the peri-infarction zones showed augmented Tc-99m PPi content.

Finally, Fig. 4 exhibits the findings in one Group C animal, in which a subendocardial myocardial infarction was identified after 7 hr of LAD occlusion. It is evident that maximal Tc-99m PPi uptake occurred in the epicardial area at risk (AR) where myocardial flow was depressed to half the nonischemic value but where cells remained viable as assessed by TTC stain (see discussion).

DISCUSSION

Animal investigations (*1,8,9,15*) have suggested that ischemic but viable myocardial cells may have increased avidity for Tc-99m PPi. Ischemic myocardial cells were identified by microsphere flow, histology, and/or muscle-enzyme criteria. These data, however, were obtained for the most part in animals with established transmural cardiac infarcts where myocardial ischemic territories appear to be relatively minor (*3*), so the uptake in seemingly viable areas may have been due to leaching of tracer from the infarcted tissue or to admixture of infarcted and nonischemic myocardial cells.

In three of the above investigations (*1,8,9*), in dogs with myocardial infarctions of durations longer than 24 hr, Tc-99m PPi concentrations five to ten times that found in nonischemic tissues were detected in peri-infarction areas. Importantly, these peri-infarction areas had either: (a) regional flows >60% of nonischemic flows (*8,9*), and thus tissue perfusion levels unassociated with myocardial necrosis (*14*), or (b) had increased intensity of succinic dehydrogenase on staining by a tetrazolium equivalent of TTC (*1*).

In the animals in Groups B and C of our investigation, we examined Tc-99m PPi sequestration in infarcted and peri-infarcted regions at 6–7 hr after LAD ligation. In Group B animals, we found that when the radiopharmaceutical is not flow-limited (1 hr reperfusion), both infarcted cardiac tissue and the 5–10-mm rim around it accumulated Tc-99m PPi equally. In contrast, in Group C animals, neither the infarcted zone nor the peri-infarcted tissue had increased radiophosphate

content. The results in Groups B and C are therefore directionally similar to those reported previously in dogs with infarcts of longer duration (*1*,*8*,*9*). It is likely that with increasing collateral flow at 24 and 48 hr, the absolute content of Tc-99m PPi in Group C animals may have increased to the levels found in the aforementioned investigations (*1*,*8*,*9*).

These results prompted us to perform studies with short-lasting LAD ligations (40 min), followed by reperfusion, in accordance to the Reimer-Jennings model (*3*). We acknowledge that this model applies strictly to circumflex ligations. However, other investigators (*4*) have found that transmural necrosis from LAD occlusions is topographically similar to that from circumflex artery occlusions.

In the six animals with 40-min LAD ligation and 6-hr reperfusion, we found that the epicardial myocardium that is transiently ischemic by microsphere flow: (a) is capable of exhibiting increased flow on reperfusion, (b) shows normal TTC staining, and (c) nevertheless has a twofold increase in Tc-99m PPi accumulation relative to nonischemic muscle. This may give a faint uptake in external imaging. However, Marcus et al. (*9*), using a ventricular phantom, determined that if a 9.5-g segment of myocardium contains twice the normal concentration of Tc-99m PPi, scintigrams of the phantom will show such areas.

We recognize that our index of cardiac tissue viability was indirect, i.e., TTC staining. This is a *histochemical* rather than a histologic criterion. Indeed, it is entirely possible that in the TTC-staining areas in the 40-min occlusions, dead cardiac cells responsible for increased Tc-99m PPi accumulation may have gone undetected. This, however, appears unlikely. The investigations of Lie et al. (*10*) and of Fishbein et al. (*11*) have established that tissues showing TTC staining generally give eight microscopic and electron-microscopic evidence of tissue viability. Furthermore, four investigations during the past year (*16–19*) used TTC staining to evaluate cardiac necrosis and the effects of therapies aimed at salvaging ischemic myocardium.

Our findings appear to support the observations in patients with unstable angina pectoris reported by Olson et al. (*20*), who showed that patients with unstable angina and *no* evidence of myocardial necrosis (by EKG or CPK enzymatic determinations) at the time of diagnosis nevertheless had 3+ diffuse Tc-99m PPi scintigrams. Our observations suggest that they may have been imaging large areas of severely ischemic tissue. Indeed, their angina patients with positive scintiscans had a markedly decreased 2-yr survival.

Furthermore, our data demonstrate that if the course of acute myocardial infarction is altered early by the restoration of adequate blood flow, Tc-99m PPi accumulates in ischemic but viable cells as well as in necrotic cardiac tissue. Thus the use of Tc-99m PPi in assessing infarct size following interventions aimed at re-establishing perfusion during acute myocardial infarction (*21*,*22*) may lead to an overestimation of the area of necrosis by including transiently ischemic tissue. This factor would be a function of the duration of increased avidity of transiently ischemic cells for Tc-99m PPi and of the timing of radiophosphate imaging after restoration of coronary flow.

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