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Localization of ^{99m}Tc-labeled Pyrophosphate and Calcium in Mycoardial Infarcts after Temporary Coronary Occlusion in Dogs[1] (39920)

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Technetium-99m-labeled phosphates accumulate in areas of necrotic myocardium following acute myocardial infarction (1-3) and have attracted considerable attention because of their potential clinical use for the diagnosis and/or quantitation of myocardial infarction and for the evaluation of myocardial injury associated with cardiac surgery. Imaging with technetium-99m-labeled pyrophosphate (^{99m}Tc-PYP) has provided both sensitive and specific confirmation of myocardial infarction diagnosed on the basis of standard ECG and enzyme changes in man (4-7). Also, infarct *localization* by imaging has closely agreed with ECG localization (4-6).

The present study (8) was done to determine whether ^{99m}Tc is excluded from ischemic but still reversibly injured myocardium and to determine the relation between tissue uptake of ^{99m}Tc and tissue calcium content.

Materials and methods. Mongrel dogs of either sex weighing 7-28 kg were anesthetized with sodium pentobarbital, intubated, and ventilated with room air with a Harvard model 1063 respirator at a rate of 300 ml/min/kg. Lead II of the ECG was monitored on a Brush Model 440-4 channel recorder. Catheters were placed in the saphenous artery (to monitor blood pressure) and vein. The heart was approached through a thoracotomy in the fourth left interspace. The circumflex branch of the left coronary artery was isolated 1.0-2.0 cm from the aortic root.

Periods of 15 and 40 min of temporary coronary occlusion were produced by tightening a snare around the circumflex artery. These two periods of injury were studied because: (i) Fifteen minutes of coronary occlusion is completely reversible in that necrosis does not occur subsequent to reperfusion (9); and (ii) 40 min of occlusion with reperfusion produces necrosis (9, 10) and marked calcium accumulation (11-13) in cells of the subendocardial myocardium. Following ischemic injury, coronary blood flow was restored by removing the snare. Dogs which developed ventricular fibrillation at this time were defibrillated, if possible, by one to four external countershocks of approximately 100 W-sec each. Ten minutes after institution of reflow, 25 mCi of ^{99m}Tc-PYP was injected via the left atrium. Thirty minutes later hearts were excised for *in vitro* evaluation of ^{99m}Tc and Ca^{2+} uptake.

The circumflex artery was opened to determine the point of occlusion in all dogs and to rule out arterial thrombi. Each heart was opened and the septum and left ventricle were laid out flat under a pinhole collimator for imaging with a gamma camera. ^{99m}Tc-PYP radioactivity was determined from identical areas of interest in ischemic and nonischemic myocardium and image ratios were calculated. The LV (including septum) was then frozen in isopentane precooled with liquid nitrogen, and samples of subendo-, mid-, and subepicardial muscle from the ischemic posterior wall and samples of nonischemic muscle were cut by hand from the frozen hearts. Each section was weighed and ^{99m}Tc radioactivity was determined in a gamma well counter. Each sample was then extracted with 0.75 *N* nitric acid, and Ca^{2+} content was deter-

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mined on a Jarrell-Ash atomic absorption flame spectrophotometer using methods described previously (11).

Counts per second per gram of technetium-99m in each section were converted to sample/nonischemic mean ratios by dividing the counts per second per gram in each sample by the counts per second per gram in the combined nonischemic sections.

Results. Twenty-four dogs underwent proximal circumflex artery occlusion. Eight dogs died from ventricular fibrillation (VF) which was most frequent within the first 10 min after occlusion or at the time of reperfusion. Sixteen dogs survived and provide the data presented. Attempts at defibrillation were not successful when VF occurred during occlusion but reflow VF was successfully terminated in six dogs which are included among the 16 survivors.

Identification of myocardial infarcts with ^{99m}Tc-PYP areas-of-interest image ratios. Area-of-interest ratios of ^{99m}Tc localization are listed in Table I. None of the six dogs subjected to 15 min of coronary occlusion showed significant ^{99m}Tc concentration.

Of 10 dogs with 40-min occlusions, six showed increased technetium localization demarcating the area of myocardial injury but four dogs showed no significant uptake on imaging. The magnitude of uptake was not related to the presence or absence of VF and defibrillation.

Transmural distribution of technetium-99m and calcium. Multiple samples of nonischemic myocardium from the 33 dogs studied showed no transmural or regional gradients of ^{99m}Tc of Ca^{2+}. Nonischemic samples were therefore pooled and the overall ^{99m}Tc radioactivity per gram was normalized to 1.0 in order to calculate ischemic/nonischemic ^{99m}Tc ratios. Individual small samples of nonischemic myocardium from each heart had ^{99m}Tc ratios which

TABLE I. TECHNETIUM RATIO AND Ca^{2+} IN THE BLOCK OF MYOCARDIUM CONTAINING THE POSTERIOR PAPILLARY MUSCLE AFTER TEMPORARY CORONARY OCCLUSION.

		Inner[a]		Mid[a]		Outer[a]		
Group	Dog No.	^{99m}Tc ratio	Ca^{2+}	^{99m}Tc ratio	Ca^{2+}	^{99m}Tc ratio	Ca^{2+}	AI[b] Image ratio
15-min temporary occlusion	2208 (DF)[c]	2.0	0.7	1.4	1.0	1.5	0.8	0.7
	2219	1.0	–	0.7	–	0.6	–	0.6
	2225	2.2	0.8	1.0	0.4	1.4	0.7	1.2
	2245	1.1	1.5	0.8	1.0	0.6	0.8	1.2
	2264 (DF)	1.9	0.5	1.1	0.5	1.4	0.4	1.3
	2274	0.9	0.8	0.7	0.9	0.7	0.6	1.2
Mean		1.5	0.9	1.0	0.8	1.0	0.7	1.0
± SE		0.2	0.2	0.1	0.1	0.2	0.1	0.1
40-min temporary occlusion	2195 (DF)	376	8.3	148	4.9	4.6	0.8	55
	2196	1237	–	820	–	2.4	–	53
	2198	967	3.0	961	1.9	24	1.1	49
	2243 (DF)	1.5	1.1	0.6	0.8	0.7	0.8	1.3
	2258	28	2.0	2.6	1.3	0.7	1.2	4.7
	2268	1.6	1.7	1.0	1.0	0.6	0.9	1.1
	2290 (DF)	2.2	0.9	1.0	0.7	0.9	0.7	2.4
	2293 (DF)	795	6.0	723	3.9	3.3	0.7	28
	2302	1.7	0.7	0.8	0.7	1.5	0.5	2.0
	2303	27	2.5	3.7	0.9	–	0.5	11
Mean		344[d]	2.9[d]	266	1.8	3.2	0.8	21[d]
± SE		151	0.9	126	0.5	1.8	0.1	7

[a] Inner, mid, and outer = subendo-, mid-, and subepicardial thirds of the transmural block of tissue containing the posterior papillary muscle. The ^{99m}Tc ratio is the ratio of counts per second per gram in the ischemic sample to counts per second per gram in pooled samples of nonischemic myocardium. Ca^{2+} is in micromoles per gram wet weight. Mean Ca^{2+} content of nonischemic muscle in the 15-min group was 0.63 ± 0.03 μmole/g and in the 40-min group was 0.80 ± μmole/g.

[b] AI = area-of-interest ratios obtained from counts of equivalent ischemic and nonischemic areas from the image obtained *in vitro* from the open left ventricle.

[c] DF = defibrillated (see text).

[d] $P < 0.05$ for ischemic vs pooled nonischemic sections.

ranged from 0.2 to 2.5. The calcium content of nonischemic muscle varied among the experimental groups from 0.63 to 1.0 μmole/g wet weight.

Following 15 min of temporary occlusion (Table I), ^{99m}Tc uptake did not exceed 2.5 in any tissue samples. Myocardial calcium was 1.5 μmole/g in one sample but did not exceed 1.0 μmole/g in any other sample.

Following 40 min of temporary coronary occlusion, technetium-99m concentration was highly variable among dogs. In those dogs with significant ^{99m}Tc accumulation, there was a transmural gradient with greatest uptake in the subendocardial myocardium (Table I). Thus the transmural distribution of ^{99m}Tc localization correlated well with the subendocardial distribution of necrosis which occurs following 40-min occlusions (9). A transmural calcium gradient was observed in the same dogs. As with ^{99m}Tc, Ca^{2+} showed a decreasing gradient of concentration from subendocardium to subepicardium. The correlation between sample ^{99m}Tc uptake and calcium content was imprecise although samples with high calcium content showed large ^{99m}Tc ratios. Samples from four of the 10 dogs in this group showed no ^{99m}Tc uptake. In three of these four dogs expected to have irreversible injury in the subendocardial myocardium, low ^{99m}Tc ratios were associated with normal or only slightly elevated calcium contents.

Discussion. Previous studies have shown that ^{99m}Tc-labeled phosphates can be used to identify myocardial infarcts in human patients (2, 4–7) and in experimental animals with coronary occlusions (1, 3, 4, 8, 14–20). These agents are accumulated within the dead tissue and thus produce positive areas of increased concentration on cardiac images. This positive uptake offers potential advantages over negative-imaging agents which depend on isotope uptake by normal myocardium. Absence of uptake with the latter agents may indicate scarring or ischemia with or without necrosis and are thus not diagnostic of acute myocardial infarction.

The specificity of technetium-99m-labeled phosphate accumulation as an index of irreversible ischemic cell injury, the mechanism, the limiting features, and the quantitative value of uptake are of considerable interest. It has been suggested (1) that ^{99m}Tc-labeled phosphates, which are well known for their capacity to bind to bone, are associated with the calcium phosphate granules which develop in mitochondria of irreversibly injured myocardial cells following ischemia (11–13). The present study was done: (i) to correlate the distribution of technetium accumulation with the usual distribution of reversible or irreversible ischemic cell injury following 15 or 40 min of circumflex artery occlusion; and (ii) to compare the myocardial distributions of ^{99m}Tc-labeled pyrophosphate and Ca^{2+} following ischemic injury.

The location and amount of necrosis following circumflex coronary occlusions of 15- or 40-min duration has been evaluated in large numbers of dogs previously studied in our laboratory. Necrosis was not evaluated histologically in the present study in order to maximize the tissue available for ^{99m}Tc and Ca^{2+} determinations and because the distribution of necrosis is difficult to evaluate at the very early times of sacrifice. Based on our previous studies, 15 min of ischemia is a completely reversible injury in that necrosis does not develop following reperfusion (9). Conversely, 40 min of temporary circumflex occlusion always causes some irreversible injury evidenced by subendocardial necrosis 24 or more hr after reperfusion (9, 10, 21, 22). The amount of necrosis from this injury varies among dogs from focal subendocardial necrosis to homogeneous subendocardial necrosis with focal midmyocardial and, rarely, subepicardial extension. The results of these previous studies allow prediction of the occurrence and approximate location of irreversible cell injury and provide the basis for evaluation of ^{99m}Tc and calcium concentration data.

Myocardium which had been transiently ischemic but was reversibly injured by 15 min of coronary occlusion showed no increased ^{99m}Tc localization. The transmural distribution of ^{99m}Tc concentration in dogs with 40 min of ischemia (Table I) also suggests that reversibly injured myocardium did not accumulate ^{99m}Tc. Subepicardial ^{99m}Tc concentration was absent in seven of

10 dogs. The slightly increased subepicardial ^{99m}Tc ratios in the remaining three dogs were most likely the result of focal subepicardial cell death which occasionally does occur following 40-min occlusions.

Although false positive results from ^{99m}Tc were not documented in this study, negative results, i.e. lack of ^{99m}Tc uptake, were observed in myocardial samples from four dogs in which irreversible injury almost certainly was present.

In 151 consecutive dogs subjected to 40 min of ischemia and several days of reperfusion, all animals have exhibited necrosis in the subendocardial myocardium (9, 10, 21, 22, and unpublished observations). Thus, it seems very unlikely that the four dogs in the present experiment with no ^{99m}Tc uptake had no necrosis. On the other hand, calcium was not markedly elevated in the tissue with no ^{99m}Tc uptake, a fact which suggests that the injury was not as severe as usual or that it was not associated with the expected calcification of the reperfusion model (11-13). We suspect that the lack of increased ^{99m}Tc uptake in the subendocardial zone observed in 4 of 10 animals, in this experiment, is related to the lack of calcification and not to the other possible explanations of no uptake: (i) the absence of necrosis, (ii) poor perfusion, or (iii) poor quality ^{99m}Tc-labeled pyrophosphate. Inadequate reperfusion is probably not the explanation because previous studies have demonstrated uniform reperfusion following 40-min occlusions (23, 24).

The site(s) and mechanism of ^{99m}Tc localization in myocardial infarcts have not been established. ^{99m}Tc-labeled pyrophosphate has a well-known predilection for bone where it is postulated to be associated with calcium phosphate. Reperfusion of irreversibly injured cells after 5-20 min of reflow is associated with massive myocardial calcium accumulation (11-13) and the simultaneous development of mitochondrial calcium phosphate granules (13, 25). Bonte's group postulated that ^{99m}Tc might be associated with these granules in areas of myocardial infarction (1).

This hypothesis was tested in a general way by comparing the myocardial calcium content with the technetium-99m ratio in each sample. Precise stoichiometric correlation between ^{99m}Tc uptake and tissue calcium concentration was not observed. This lack of correlation may be related to the facts that (i) the actual molar concentrations of ^{99m}Tc and pyrophosphate, (ii) the cellular and subcellular distribution of calcium and the proportion present in the form of mitochondrial calcium phosphate granules, and (iii) the calcium-binding characteristics of ^{99m}Tc-PYP among different preparations of the pharmaceutical are unknown. Nevertheless, in hearts from the 40-min temporary occlusion studies samples with normal calcium contents usually showed low ^{99m}Tc ratios and samples with ^{99m}Tc ratios above 25 always showed increased calcium content. Also, transmural technetium-99m gradients usually were associated with similar transmural calcium gradients. Three samples from the 40-min group showed significant ^{99m}Tc uptake despite relatively low calcium uptake. These three samples were from mid and subepicardial myocardium where irreversible injury most likely was focal. Focal cell calcification might be sufficient to cause significant ^{99m}Tc localization, yet insufficient to raise total tissue calcium content.

Summary. The location and magnitude of ^{99m}Tc concentration was assessed following 15 or 40 min of temporary coronary occlusion to evaluate uptake with respect to irreversible vs reversible ischemic cell injury and with respect to the presence or absence of increased myocardial Ca^{2+} content. ^{99m}Tc did not accumulate in any samples after 15 min of ischemia (reversible injury). ^{99m}Tc uptake was observed in 6 of 10 dogs following 40 min of occlusion and the high ^{99m}Tc ratios were associated with massive tissue calcification. The results indicate that ^{99m}Tc accumulation is specific for irreversible ischemic injury and is associated with tissue calcification.

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