Localization of Radiolabeled Cardiac Myosin-Specific Antibody in Myocardial Infarcts

Comparison with Technetium-99m Stannous Pyrophosphate

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SUMMARY The myocardial uptake of radioiodinated (Fab')2 fragments of antimyosin antibody [131I-(Fab')2] was compared with simultaneously administered 99mTc-pyrophosphate (Tc-PYP) in dogs undergoing coronary occlusion for 24 (N = 6) or 72 hours (N = 5). Relative concentrations of both agents in normal and infarcted myocardium were related to regional blood flow as determined by distribution of 99mTc-labeled microspheres in the same animals.

METHODS HAVE BEEN DEVELOPED to localize and size acute myocardial infarcts with agents that are selectively sequestered in areas of myocardial damage. The radiopharmaceuticals employed in early experimental studies in animals included 203Hg-chlormerodrin, 1 209Hg-mercurifluorescein, 2 and 67Ga-citrate. 3 More recently, localization and sizing of acute myocardial infarcts have been accomplished with technetium-99m tetracycline, 4 technetium-99m glucoheptonate, 5 and various technetium-99m labeled phosphate compounds. 6,7 These radiopharmaceuticals, particularly technetium-99m pyrophosphate, have been successfully employed clinically for infarct imaging with a gamma scintillation (Anger) camera. 8-11 We have recently reported the development of a new approach to localization of myocardial infarcts using radio-labeled antibodies to cardiac myosin that gain entry into damaged myocardial cells and bind specifically to myosin rendered accessible to extracellular macromolecules. 12 The purpose of the present study was to compare simultaneously the distribution of purified (Fab')2 fragments of antimyosin antibody with that of 99mTc-pyrophosphate in dogs undergoing experimental myocardial infarction. Relative concentration of both agents in damaged and normal myocardium was compared to relative regional flow values in the same animals.

Methods

Preparation and Purification of 131I-Labeled Antimyosin (Fab')2 Fragments

The methods for isolation of myosin from canine left ventricular myocardium, immunization of rabbits with purified canine myosin, and testing of antisera for antibody activity were as previously described. 13 (Fab')2 fragments of rabbit antibody specific for canine cardiac myosin were obtained by peptic digestion and purified by affinity chromatography utilizing a myosin-Sepharose immunoadsorbent as previously described. 14

Radiiodination of (Fab')2 fragments of antimyosin antibody was performed by the lactoperoxidase procedure of Marchalonis 15 with carrier free Iodine-125. The specific activity of radioiodinated antimyosin (Fab')2 fragments was 130 Ci/mM, assuming a molecular weight of 100,000 daltons for (Fab')2.

Experimental Infarct Model

Anterior myocardial infarction was produced in 15 dogs utilizing techniques previously described. 16 Animals were anesthetized with intravenous pentobarbital (30 mg/kg) and ventilated with a Harvard Respirator at an FiO2 of 0.4. Following a left thoracotomy, the heart was suspended in a pericardial cradle to which 2-0 Mersilene ligatures were placed around sequential branches of the left anterior descending coronary artery. These branches were then serially ligated at 2-3 minute intervals until approximately 1/3 to 1/2 of the anterior surface of the left ventricle appeared cyanotic. Coronary venous branches were left intact. Transient ventricular ectopic activity was occasionally encountered and treated with a 30 mg intravenous bolus of lidocaine.

A flared-end polyethylene cannula was inserted into the left atrium via a stab wound in the left atrial appendage and held in place with a purse-string suture. The thoracotomy was then closed and the animals allowed to recover. The exteriorized atrial cannula was kept patent with frequent flushes with heparinized saline. All dogs appeared active and healthy during the subsequent study period.

Regional Blood Flow Measurements

Relative regional myocardial blood flow was measured in all dogs at the end of the occlusion period with strontium-85 labeled 15 ± 5 μ carbonized microspheres as previously...
described. Microspheres were obtained as 1 mCi of nuclide suspended in 10 ml of 10% Dextran with Tween 80 added to avoid clumping. Prior to withdrawing a dose, the vials containing microspheres were dispersed in a Vortex Mixer for 5 minutes and then agitated in an ultrasonic bath for another 20 minutes. The dose injected was $4 \times 10^6$ microspheres. At the time of sacrifice, multiple myocardial specimens from ischemic and nonischemic zones were counted in a Nuclear Chicago gamma well scintillation counter as described below.

Experimental Protocol

In a group of six dogs undergoing myocardial infarction in the manner described above, 100 $\mu$Ci of (Fab')$_2$ fragments of antimyosin antibody labeled with $^{125}$I-$^{125}$I-(Fab')$_2$ was injected intravenously four hours after coronary occlusion. The animals were allowed to recover and 20 hours later 3 mCi of $^{99m}$Tc on 1.75 mg % stannous pyrophosphate (Tc-PYP) carrier was injected intravenously. Fifty-five minutes after Tc-PYP administration, $^{85}$Sr-labeled microspheres were injected into the left atrium and 5 minutes later the animals were sacrificed. Multiple transmural myocardial specimens (0.5 to 1.0 g) from infarcted and normal myocardium were obtained. These samples were divided into endocardial and epicardial halves, weighed, and counted in a gamma well scintillation counter at the appropriate energy windows for the three nuclides with correction of counts in each channel for spill from other channels. To insure accuracy of counts, the samples were recounted 72 hours later when $^{99m}$Tc activity had decayed to less than 0.1% of its activity at the time of injection. Relative uptakes of Tc-PYP and $^{125}$I-(Fab')$_2$ fragments of antimyosin antibody were calculated as ratios of uptake in infarcted regions to that present in nonischemic posterior left ventricular myocardium. Relative regional blood flow in these samples was expressed as percent of nonischemic posterior wall flow.

In another five dogs, a similar protocol was carried out except that the animals were sacrificed 72 hours after coronary occlusion. $^{125}$I-(Fab')$_2$ was again administered four hours after occlusion, but Tc-PYP was given 71 hours after occlusion. Fifty-five minutes after Tc-PYP administration, microspheres were injected and five minutes later the animals were sacrificed. The longer time period of occlusion was chosen since both antimyosin (Fab')$_2$ fragments and $^{99m}$Tc-PYP have been shown to increase in concentration in damaged myocardium over this range of increasing duration of occlusion.

Kinetic Studies

In another four dogs, serial blood samples after $^{125}$I- or $^{131}$I-antimyosin (Fab')$_2$ administration were obtained during a 72-hour period following coronary occlusion. Blood clearance of the tracer was plotted and ratios of concentration of antimyosin (Fab')$_2$ in normal myocardium to blood at 72 hours were calculated.

Results

Relative localization of $^{125}$I-(Fab')$_2$ and Tc-PYP in relation to relative regional myocardial blood flow in all myocardial samples ($N = 130$) from the group of dogs occluded for 24 hours is shown in figure 1. An inverse exponential relationship between antimyosin (Fab')$_2$ localization and regional blood flow is evident ($r = -0.62$). The greatest uptake of antimyosin (Fab')$_2$ was observed in subendocardial layers of the center of the infarct where regional flow was most severely impaired. In contrast, relative uptake of Tc-PYP in these same regions bore no simple relationship to regional flow (fig. 1). Figure 2 relates the relative uptake of both tracers to regional flow in another manner. In myocardial samples where flow was reduced to 1–10% of normal flow, relative $^{125}$I-(Fab')$_2$ uptake was 4.8 ± 0.4 (SEM) ($P < 0.001$). Relative uptake was 3.9 ± 0.4 ($P < 0.001$) in specimens where flow was reduced to 11–30% of nonischemic flow and 3.0 ± 0.3 ($P < 0.001$) in regions where flow was reduced to 31–50% of normal. In areas where flow was only moderately (51–80% of normal) or slightly (< 81% of normal) reduced, antimyosin (Fab')$_2$ uptake progres-
sively diminished to 2.2 ± 0.1 and 1.5 ± 0.8, respectively.

After 24 hours of occlusion, Tc-PYP uptake in infarcted tissue was greater than 125I-(Fab')2 uptake in the same myocardial samples (fig. 2). However, the mean ratio of Tc-PYP uptake in areas of greatest flow reduction to normal uptake (11.6 ± 1.8) was not significantly different from uptake (9 ± 3) in regions where flow was only minimally reduced. Maximal localization of 99mTc-PYP was observed in samples from the periphery of the infarct where flow was decreased to 11-30% of normal after occlusion.

As summarized in figures 3 and 4, qualitatively similar findings were observed in the dogs undergoing coronary occlusion for 72 hours. In this group, there was a strong inverse exponential relationship between 125I-(Fab')2 uptake and regional blood flow ($r = -0.80$). As was the case for 24-hour experiments, no such simple correlation between flow and Tc-PYP uptake was found. Maximal 125I-(Fab')2 uptake was again observed in the infarct center where flow was most severely impaired (fig. 4). Mean relative uptake in this region after 72 hours, however, rose to 14.9 ± 0.9, significantly higher than the mean ratio in this zone in 24-hour occluded dogs. Comparable increases in 125I-(Fab')2 concentration were observed in all zones of flow reduction. As seen in 24-hour occluded dogs, maximal localization of Tc-PYP in dogs occluded for 72 hours was found in areas of moderate flow reduction (31-50% of normal), with relative uptake (31.9 ± 3.5) only slightly higher than observed after 24 hours of occlusion. Tc-PYP uptake in the lowest flow area in the center of the infarct (12.8 ± 2.6) was again not significantly different from uptake in regions of only minimal flow reduction (14.0 ± 1.9). Localization of antimyosin (Fab')2 was significantly greater in subendocardial than in subepicardial regions of infarcted myocardium, whereas Tc-PYP localization was significantly greater in subepicardial zones. In the experimental infarction model employed in these studies, regional flow to subendocardial regions is more severely impaired.

Figure 5 shows the blood clearance of radiolabeled antimyosin (Fab')2 fragments in four experimentally infarcted dogs. Blood activity (cpm/g) was significantly higher at 24 hours post-Fab'2 injection. Each value is expressed as percent of initial blood activity determined 15 minutes after injection. Four different symbols are used to distinguish values for each individual animal.

**Discussion**

Radionuclide-labeled substances sequestered by acutely infarcted myocardium may provide a more direct method for detection and localization of infarction and for determining its size. Localization of acute myocardial infarction has been achieved with a number of compounds labeled with technetium-99m including 99mTc-pyrophosphate, 99mTc-tetracycline and 99mTc-glucophenolate.4-14 99mTc-pyrophosphate has been the most widely employed of these radiopharmaceuticals for localizing myocardial infarcts in patients11-14 and has demonstrated a high degree of sensitivity. Results from recent studies have demonstrated a close correlation between Tc-PYP uptake with histological...
infarct size\textsuperscript{20, 21} and with the degree of creatine phosphokinase depletion.\textsuperscript{14} The mechanism of uptake of \textsuperscript{99m}Tc-PYP appears to be entry through injured myocardial cell membranes and subsequent binding to intracellular components.\textsuperscript{19, 20} Details of the mechanism by which Tc-PYP accumulates in damaged myocardial cells have not been fully worked out, but it has been shown that uptake of Tc-PYP occurs in infarcted myocardial tissue in which there is histological evidence of cell necrosis and intracellular calcium accumulation.\textsuperscript{18, 20} Following experimental coronary occlusion in dogs, analysis of infarcted myocardial tissue has shown calcium deposits in necrotic muscle cells predominantly located in the outer peripheral regions of one-day-old infarcts, in the form of apatite-like crystals in mitochondria.\textsuperscript{22}

In the present studies, myocardial uptake of (Fab')\textsubscript{2} fragments of antimonyosin antibody was compared with uptake of Tc-PYP in dogs undergoing coronary occlusion for 24 or 72 hours. The rationale for using \textsuperscript{123}I-(Fab')\textsubscript{2} fragments in these experiments was that these molecules have two-thirds the molecular weight of intact antibody, which may allow for greater intracellular entry through damaged cell membranes, but maintain binding advantages of bivalency. We have previously shown that intravenously administered radiiodine-labeled antimonyosin antibody or its (Fab')\textsubscript{2} fragments are selectively localized in infarcted myocardium of dogs.\textsuperscript{18} Antimonyosin antibody presumably gains entry into myocardial cells after ischemia-induced increase in membrane permeability, which also allows for the egress of macromolecules such as creatine kinase after acute infarction. Maximal uptake of antimonyosin antibody or its (Fab')\textsubscript{2} fragments was observed in subendocardial layers of the center of the infarct, with decreasing uptake toward the infarct periphery as determined by gross inspection. Since this distribution pattern of antimonyosin antibody differed considerably from that previously described for Tc-PYP,\textsuperscript{19, 20} we sought to determine the uptake of both agents in the same heart after coronary occlusion, with simultaneous assessment of regional blood flow.

Data from the present study show that maximal uptake of Tc-PYP was in the periphery of the infarct where regional flow is reduced to an intermediate extent, confirming observations by other investigators.\textsuperscript{19, 20, 23, 24} On the other hand, the greatest concentration of antimonyosin (Fab')\textsubscript{2} in the same hearts was seen in the center of the infarct where flow was most severely impaired (1–10% of normal flow). Localization of antimonyosin (Fab')\textsubscript{2} was inversely related to flow, whereas Tc-PYP uptake ratios were similar in central zones of severe flow reduction and in the outermost region of the infarct where flow was moderately reduced.

The differences in myocardial distribution of these two agents in ischemic myocardium are probably related to differences in kinetics of exit from the blood pool. It has been shown that Tc-PYP is cleared from the blood in a biexponential fashion.\textsuperscript{26} The clearance half-time of exponent I is 13.6 minutes and represents bone uptake. Exponent II had a mean blood clearance half-time of 380 minutes and represents urinary excretion. Four hours after intravenous administration, an average of only 1.9% of the injected dose per liter of blood was demonstrable. Because of this rapid disappearance from the blood pool, there is limited exposure time for the radionuclide to enter and be sequestered in areas of severely diminished flow. On the other hand, preservation of greater flow to peripheral zones of infarction provides delivery of the agent to intracellular sites of deposition in damaged cells.

In contrast, \textsuperscript{123}I-(Fab')\textsubscript{2} has a much longer half-life in the blood, giving a significantly higher blood background radioactivity at 24 hours than Tc-PYP. However, by 72 hours blood levels of \textsuperscript{123}I-(Fab')\textsubscript{2} are substantially reduced and are only two times greater than normal myocardium. Because of the longer half-life in the blood, there is sufficient time for antimonyosin (Fab')\textsubscript{2} to accumulate in areas of low flow where myocardial cellular damage is most pronounced. There appears to be sufficient flow even in regions of greatest infarction to allow for eventual equilibration so the myosin specific antibody concentration is probably proportional to the amount of accessible myosin, e.g., the number of myocardial cells having permeable membranes. Decrease of antibody concentration in blood with time is sufficiently slow to permit this equilibration.

Because of these differences in kinetics \textsuperscript{123}I-(Fab')\textsubscript{2} was injected four hours after coronary occlusion to allow longer equilibration times for radionuclide uptake in 24 or 72 hour occluded animals. Tc-PYP was injected one hour prior to sacrifice in both groups of animals because of its more rapid equilibration time.

Another interesting finding in the present study was that the Tc-PYP uptake ratio, compared with normal myocardium, was 9:1 in areas of only slight flow reduction (90% of normal). Antimonyosin (Fab')\textsubscript{2} uptake in these same samples was not significantly greater than background activity. This difference suggests that Tc-PYP might localize in ischemic as well as infarcted myocardium. On the other hand, this zone of minimal flow reduction may contain heterogeneous cell populations in which severely or irreversibly damaged cells are situated adjacent to normal cells. Pyrophosphate may be taken up in high concentration by these severely injured cells, particularly since nutrient flow to this region is well preserved allowing for greater delivery of the nuclide than can be achieved in central areas of infarction. The issue of whether Tc-PYP is taken up by ischemic as well as by necrotic tissue is not entirely settled. Zaret and coworkers,\textsuperscript{24} using a closed chest canine infarct preparation, found maximal Tc-PYP activity in myocardial samples showing only mild reduction in CPK and 43K uptake, whereas Botvinick et al. showed a significant negative correlation between tissue Tc-PYP activity and CPK content.\textsuperscript{25} In the present study, antimonyosin (Fab')\textsubscript{2} uptake was significantly greater in subendocardial than subepicardial layers of ischemic myocardium, whereas Tc-PYP localization was greater in subepicardial regions.

The results of this study suggest that antimonyosin (Fab')\textsubscript{2} may be potentially useful as an imaging agent for detection and localization of myocardial infarcts, but several problems still need to be overcome. Blood levels of the radionuclide remain above background for at least 72 hours after administration. In addition, infarct-to-normal uptake ratios of antimonyosin (Fab')\textsubscript{2} are not optimal until 72 hours after injection precluding rapid sequential studies. A major theoretical advantage of this approach is the specificity of the antibody for a cardiac intracellular protein. The immunologic problems related to the injection of proteins from one species into another species do not appear to be a serious
limiting factor in the application of this technique to infarct imaging. Only microgram quantities of antimyosin antibodies are being administered and elimination of the Fc fragment with the use of (Fab')2 fragments avoids complement-mediated immunologic reactions.

In conclusion, this study shows that (Fab')2 fragments of antimyosin antibody localize in infarcted myocardium in a manner inversely proportional to regional blood flow. The ratios of uptake in infarct to normal left ventricle (15–20:1) are not as high as those seen with Tc-PYP, but greatest 123I-(Fab')2 localization occurs in regions of lowest flow in subendocardial layers of central zones of infarction. In contrast, regional localization of Tc-PYP is greatest in subepicardial layers of the infarct periphery. The differences in the uptake patterns of Tc-PYP and radiiodinated antimyosin (Fab')2 fragments appear to be related in part to the difference in the kinetics of exit of each agent from the blood pool.

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Localization of radiolabeled cardiac myosin-specific antibody in myocardial infarcts. Comparison with technetium-99m stannous pyrophosphate.

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