Experimental Myocardial Infarct Imaging following Intravenous Administration of Iodine-131 Labeled Antibody (Fab')2 Fragments Specific for Cardiac Myosin

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SUMMARY Canine myocardial infarcts resulting from ligation of the left anterior descending coronary artery were localized in vivo by gamma scintigraphy following the intravenous injection of 131I-anti-cardiac myosin antibody (Fab')2. The anteroposterior location of the image was confirmed by demonstration of the blood pool with 99mTc sulfur colloid, and by subsequent imaging of the excised heart. The scintigram of the excised heart, following prior in vivo injection of 141Ce-microspheres, showed a region of diminished radioactivity near the apex which corresponded precisely to the region of 131I-antibody (Fab')2 uptake. Well defined areas of 131I antibody (Fab')2 activity in the region of infarction were consistently imaged at 48 hours in animals in which the ligature occluding the coronary artery was released at 5 hours; 72 hours were at times required when coronary occlusion persisted throughout the experiment. In both reflow and persistent occlusion models, the concentration of 131I antibody (Fab')2 was inversely related to blood flow as determined from microsphere distribution in the region of infarction; though in areas of equivalent flow (0 to 20% of normal) the mean concentration ratio of antibody uptake to normal tissue was 20.7 ± 2.2 in the reflow model as compared to 11.4 ± 0.7 in the persistent occlusion model. A reduction in blood flow to the affected area of < 50% of normal did not result in the production of a localized scintigraphic image.

THE APPLICATION OF RADIOPHARMACEUTICALS to imaging myocardial infarcts has been an area of considerable recent interest and activity.1-8 Of the substances tested, technetium-99m labeled phosphate compounds, especially technetium-99m pyrophosphate, have been most successful in clinical myocardial infarct imaging.9-13 However, their specificity for localization in myocardial infarcts has recently been questioned.14-16 We have recently reported the development of a new method for detection and localization of myocardial infarcts employing intravenously administered radioiodine-labeled (Fab')2 fragments of antibodies specific for cardiac myosin. In our initial experiments in a canine infarct model,17 distribution of 125I or 131I-labeled (Fab')2 fragments of antimyosin antibody in ischemic and infarcted myocardium was determined by gamma well scintillation counting. The ratios of uptake in infarct-to-normal left ventricle ranged from 15-20:1 after 72 hours of infarction and concentration of the radioiodinated (Fab')2 fragments were shown to be inversely proportional to regional blood flow, with the greatest uptake observed in endocardial layers of the central infarct zone.17, 18

Because of this high infarct-to-normal ratio, further studies were undertaken to determine if 131I-labeled (Fab')2 fragments of antomyosin antibody could be utilized for "hot spot" imaging of infarcts employing a gamma scintillation camera. In this communication we show that these observations may be extended to the imaging of myocardial infarcts utilizing the Anger camera after intravenous administration of 131I-labeled antimyosin (Fab')2 (131I-Ab(Fab')2).

Methods

Purification of Antimyosin (Fab')2 Fragments

Purification of homogeneous canine cardiac myosin, immunization of rabbits, and purification of rabbit antimyosin antibody by cardiac myosin-Sepharose immuno-adsorbent were carried out as previously described.19 Antimyosin antibody (Fab')2 fragments were prepared by pepsin digestion of the intact antimyosin antibody at 37°C for 20 hr at pH 4.5 according to the method of Edelman and Marchalonsis.20 Separation of the (Fab')2 fragments was by Sephadex-G100 column chromatography (2.5 × 90 cm).

Radioiodination of Antimyosin (Fab')2 Fragments

Antimyosin (Fab')2 fragments were iodinated with 131I by the lactoperoxidase procedure of Marchalonsis.20 For each antimyosin (Fab')2 iodination, approximately 5 mCi of 131I were covalently coupled to 500 micrograms of the antibody (104 Ci/mM).

Experimental Myocardial Infarct Models

Two canine experimental myocardial infarction (MI) models were employed.

MI produced by persistent coronary artery occlusion: Six mongrel dogs (19-22 kg) were anesthetized by intravenous pentobarbital (30 mg/kg) prior to left thoracotomy performed under sterile conditions. Progressive ligation of the confluent branches of the left anterior descending coronary artery was carried out at 2-3 min intervals until approximately 40% of the anterolateral surface of the left ventricle appeared cyanotic.17, 21 Coronary veins were left intact. Transient ventricular ectopic activity encountered occasionally was treated with an intravenous bolus injection of lidocaine (30 mg). A flared-tip catheter was inserted into the left atrial appendage via a stab wound and secured by purse-string suture. The catheter was flushed twice daily with heparinized saline. The thoracotomy was closed and the animals were allowed to recover. All dogs studied appeared active after recovery from anesthesia.

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M1 produced by 5 hour coronary artery occlusion followed by reperfusion: Another seven mongrel dogs (19–22 kg) were anesthetized and subjected to ligation of confluent diagonal branches of the left anterior descending coronary artery as described above. The ligatures were released after 5 hr. A left atrial catheter was also inserted. The thoracotomy was then closed and the animals allowed to recover.

An intravenous injection of Keflin (1 g/animal) was given to all animals.

Determination of Regional Blood Flow

Relative regional myocardial blood flow was determined by atrial administration of a bolus of $4 \times 10^6$ Cerium-141 labeled 7 to 10 μ carbonized microspheres$^{22}$ (3 M Co.) via the left atrial catheter. The microspheres were divided in 1 mCi aliquots suspended in 10 ml of 10% Dextran with Tween 80 to avoid clumping. Dogs with persistent coronary artery occlusion received $4 \times 10^6$ 141Ce-microspheres injected via the left atrial catheter 15 min prior to the termination of the experiment. Dogs subjected to reperfusion were given $4 \times 10^6$ microspheres 15 min prior to reperfusion. At the end of the experiment, hearts were excised and then imaged for 141Ce-microsphere distribution employing an Anger camera at 145 keV Ce-141 photopeak. Multiple transmural tissue samples from the anterior and posterior left ventricular walls were then obtained and relative regional flow determined by in vitro gamma well scintillation counting in a Packard Auto-Gamma Scintillation Spectrometer.

Gamma Scintigraphy

Radioisotope localization in intact animals and excised hearts was visualized utilizing an Ohio Nuclear Series 100 Anger camera equipped with a medium energy collimator. For each image of the intact animals 200,000 to 500,000 counts were collected, and 10,000 to 20,000 counts were collected for the excised hearts. 131I gamma detection was set at 365 keV 131I-photopeak and the 145 keV 141Ce photopeak was used for 141Ce-microsphere distribution imaging. In in-

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**Figure 1.** Experimental protocols: experimental myocardial infarction produced by persistent occlusion of the left anterior descending coronary artery, and occlusion of the LAD for 5 hr followed by reperfusion.

**Figure 2.** Left lateral gamma scintigram of a dog with persistent coronary occlusion at 72 hr after occlusion (a), blood pool image of the left ventricle by first pass imaging of $99m$Tc sulfur colloid administered intravenously (b), and superimposed computer enhanced isocount lines of $131I$-Ab(Fab')$_2$ hot spot and $99m$Tc-sulfur colloid cavity image (c).
tact animals, 1 mCi of $^{99m}$Tc sulfur colloid was injected into the left atrial catheter in order to localize the position of the left atrium and left ventricle. A 140 keV $^{99m}$Tc-photopeak was used to image the LV cavity by the first pass method utilizing 1 mCi $^{99m}$Tc-sulfur colloid intravenously administered.

Scintigraphic data were analyzed with a PDP-9 computer to produce an image display. Excised hearts were imaged in the lateral position. Intact animals were also imaged in the left lateral position, except in a few dogs in which anteroposterior images were also obtained.

Experimental Protocol

To a group of eight dogs with persistent coronary artery occlusion, 1 mCi of $^{131}$I-Ab(Fab')$_2$ was injected intravenously at 4 hr of occlusion. Gamma scintigraphy was performed in the left lateral position at 48 and 72 hr after antibody administration. The location of the left ventricle was determined after the administration of 1 mCi of technetium-$^{99m}$ sulfur colloid either by atrial catheter or intravenously by the first pass ventricular blood pool imaging. Fifteen minutes prior to termination, $^{141}$Ce-microspheres were injected via the left atrial catheter. Excised hearts were then imaged for $^{131}$I-Ab(Fab')$_2$ localization as well as for the $^{141}$Ce-microsphere distribution image. Multiple transmural biopsy samples were then obtained from test myocardium and from the normal posterior left ventricular myocardium, and radioactivity of each isotope determined by scintillation counting (fig. 1).

To another group of seven dogs with 5 hr of coronary artery occlusion followed by reperfusion, $^{141}$Ce-microspheres were administered via the left atrial catheter 15 min prior to reperfusion. Thirty minutes after the microsphere injection, 1 mCi of $^{131}$I-Ab(Fab')$_2$ was injected intravenously. The dogs were allowed to recover and at 48 and 72 hr after occlusion, gamma scintigraphy was performed to visualize localization of $^{131}$I-Ab(Fab')$_2$. The location of the left ventricle was determined as described above. The excised hearts were also studied as described for dogs with persistent coronary artery occlusion (fig. 1).

Statistical Analysis

Linear regression curves relating relative $^{131}$I-Ab(Fab')$_2$ uptake to regional blood flow were obtained employing statistical analysis described previously. 17, 18

Results

Examples of gamma scintigrams showing localization of $^{131}$I-Ab(Fab')$_2$ in myocardial infarcts produced by persistent coronary artery occlusion are shown in figures 2, 3, 4, and 5. In each instance, maximal and discrete localization of activity was observed in sites of infarction. Figure 2a shows left lateral scintigrams in a dog undergoing 72 hr coronary artery occlusion and demonstrates localization of the antibody in a distinct hot spot of $^{131}$I-Ab(Fab')$_2$ activity in the region of the apex of the left ventricle. The position of the left ventricle was determined by the first pass imaging of $^{99m}$Tc-sulfur colloid (fig. 2b). The superimposed computer enhanced isocount lines of the $^{131}$I-Ab(Fab')$_2$ hot spot and that of the left ventricular cavity image confirms the apical localization of the hot spot of $^{131}$I-Ab(Fab')$_2$ localization (fig. 2c).

The gamma scintigram of the excised heart of this dog also showed the anteroapical localization of $^{131}$I-Ab(Fab')$_2$ (fig. 3a) which was observed to correspond to the area cold spot in the perfusion scintigram which was obtained by imaging $^{141}$Ce-microsphere distribution (fig. 3b). The superimposed picture of scintigrams 3a and 3b demonstrates the filling in of the perfusion defect by the hot spot (fig. 3c).

Figure 4 shows gamma scintigrams of $^{131}$I-Ab(Fab')$_2$ localization in another dog 72 hr following coronary occlusion. Images obtained while the animal was intact show

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**Figure 3.** Left lateral scintigrams of the excised heart at 72 hr from a dog with persistent coronary occlusion. Anteroapical hot spot of $^{131}$I-Ab(Fab')$_2$ localization (a), which corresponds to the anteroapical perfusion defect determined by $^{141}$Ce-microsphere distribution (b); both are shown superimposed in c.
FIGURE 4. Left lateral scintigrams of two dogs with persistent coronary artery occlusion. (a and b) Area of catheter insertion is indicated by the top arrow; bottom arrow denotes urinary bladder activity. The central hot spot is the ventricular lesion (a); the apical location of the hot spot is shown in (b) by the superimposed outline of the ventricular cavity; and (c) left lateral scintigram of another dog with persistent coronary occlusion.

Three areas of discrete localization of $^{131}$I-Ab(Fab')$_2$ activity (fig. 4a). The area of increased radioactivity situated just above the bottom arrow is the location of the urinary bladder; that indicated by the top arrow represents uptake of $^{131}$I-Ab(Fab')$_2$ by the atrium at the site of catheter insertion and associated suturing; the central hot spot is the location of the left ventricular infarct. Its apical localization is demonstrated by the superimposed outline of the left ventricular cavity (fig. 4b). Figure 4c shows a left lateral scintigram of another dog with persistent coronary occlusion at 72 hr. In vivo gamma imaging of dogs with persistent coronary occlusion is feasible only when the infarct is greater than 11 g of the left ventricular mass. Gamma scintigrams of three more excised hearts showing anteroapical localization of $^{131}$I-Ab(Fab')$_2$ activity are shown in figure 5.

FIGURE 5. Left lateral images of three other excised hearts from dogs with persistent occlusions at 72 hr.

FIGURE 6. Relationship between $^{131}$I-Ab(Fab')$_2$ localization in infarcted myocardium and corresponding regional blood flow at 72 hr after coronary occlusion in six dogs. Relative $^{131}$I-Ab(Fab')$_2$ uptake and $^{85}$Ce-microsphere radioactivity were determined for each transmural myocardial sample simultaneously by gamma spectrometry. A linear curve (solid line) was obtained by utilizing $y = a + bx$ where $y = \log_{10}(\% \text{ normal blood flow})$, $x =$ relative antimyosin uptake, $a = 2.08$ and $b = -0.067$. 

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The relationship between relative $^{131}$I-$\text{Ab(Fab')}_2$ uptake and regional myocardial blood flow in transmural biopsy samples from six dogs with persistent occlusion at 72 hr (fig. 6) was inverse ($r = -0.81$). Highest ratios of antibody uptake were observed in the myocardial samples of the most severe flow reduction. Two of six dogs with persistent coronary occlusion had only moderate reduction in regional myocardial blood flow (fig. 7). The majority of the transmural samples had regional blood flow reduced no more than 50% of normal myocardial blood flow, and only two transmural biopsy samples (≈ 2 g) indicated flow reduction between 35% and 50% of normal; all other samples showed flows in excess of 50%. With such minimal compromise in flow, no hot spot localization of $^{131}$I-$\text{Ab(Fab')}_2$ was observed, even when excised hearts were imaged.

Dogs (N = 7) with coronary artery occlusion followed by reperfusion, consistently showed distinct localization of $^{131}$I-$\text{Ab(Fab')}_2$ in gamma scintigrams obtained 48 hr after occlusion. Figure 8 shows scintigrams of a dog with 5 hr of occlusion followed by reperfusion. A well defined hot spot of $^{131}$I-$\text{Ab(Fab')}_2$ activity is observed in the 48 hr gamma scintigram (fig. 8a). The apical localization of the antibody was demonstrated by the superimposition of the outline of the left ventricular cavity (fig. 8b). The same dog reimaged 72 hr after occlusion showed increased localization of $^{131}$I-$\text{Ab(Fab')}_2$ activity (fig. 8c); its apical localization is shown in figure 8d. Gamma scintigrams of the excised heart show apical localization of the radio-iodinated antibody (fig. 9a) which corresponded to the region of perfusion defect as shown by gamma scintigraphy of $^{141}$Ce-microsphere distribution (fig. 9b). Figure 9c shows the relative locations of the $^{141}$Ce-microsphere distribution image and of $^{131}$I-$\text{Ab(Fab')}_2$ localization, demonstrating that the area of anti-
left lateral and anteroposterior gamma in vivo scintigrams of another dog with occlusion followed by reperfusion at 72 hr. This infarct was sized subsequently at only 2.5 g of the myocardium. The ratio of $^{131}$I-Ab(Fab')$_2$ in infarct tissue to normal myocardium was about 30:1.

The relationship between $^{131}$I-Ab(Fab')$_2$ uptake and relative regional myocardial blood flow in seven dogs with coronary occlusion followed by reperfusion is shown in figure 12. An inverse relationship between antibody uptake and relative regional myocardial blood flow is again observed ($r = -0.79$) with highest antibody uptake (20.7 ± 2.2 [SE]) in regions of lowest blood flow (8.0 ± 1.1% [SE]).

Table 1 demonstrates the amount of the left ventricular myocardium in the test regions in relationship to the mean antimyosin (Fab')$_2$ uptake and regional blood flow from two representative dogs, one with persistent coronary occlusion and the other with reperfusion after 5 hr of coronary occlusion. In both animals, the amount of left ventricular myocardium with flow reduction greater than 50% of normal flow was approximately the same, 12.794 g in the dog with persistent coronary occlusion and 14.253 in the dog with coronary reperfusion. However, mean ratios of antibody uptake were consistently higher for the dog with coronary reperfusion.

No overt toxic effect was observed in any of the animals as a result of $^{131}$I-Ab(Fab')$_2$ intravenous administration for the duration of the experiment.

Discussion

Hot spot gamma imaging of acute myocardial infarction has been achieved by employing radiopharmaceuticals such as $^{99m}$Tc-pyrophosphate, $^{99m}$Tc-tetracycline, and $^{99m}$Tc-glucoheptonate. Of these imaging agents, $^{99m}$Tc-pyrophosphate has been used most successfully experimentally and clinically, though its specificity is not exclusive for myocardial infarcts. Localization of $^{99m}$Tc-PYP has been shown to occur in aneurysms, in skeletal muscle following cardioversion, as well as in carcinoma of the lung.
The concentration of \(^{99m}\)Tc-PYP in MI does not appear to be proportional to the level of myocardial necrosis.\(^{15}\)

The present study demonstrates the feasibility of employing a highly specific method for imaging of myocardial infarcts based on the hypothesis that damaged myocardial cells that allow leakage of macromolecules such as CK, LDH, and SGO\(^{26-28}\) also permit the reverse entry of intravenously administered radiolabeled antimyosin (Fab')\(_2\) fragments that then bind cardiac myosin, the most abundant protein in the cardiac cells.\(^{29}\) The specificity of antimyosin (Fab')\(_2\) for damaged myocardial cells has been demonstrated previously.\(^{17}\) Antimyosin antibody is not solely specific for myocardial infarction, since it will bind all damaged or necrotic myocardial cells. However, it will not localize in necrotic skeletal muscles above nonspecific adsorption levels. The present study confirms that no significant localization of antimyosin (Fab')\(_2\) occurred in canine myocardium when regional blood flow was only moderately compromised, i.e., reduced not less than 50% of normal blood flow in the majority of transmural myocardial biopsy samples. When regional myocardial blood flow was reduced by greater than 70% of normal flow, discrete localized areas of activity were demonstrable. The greatest uptake of antimyosin (Fab')\(_2\) was demonstrated in those transmural samples with greatest regional blood flow reduction. Studies employing microspheres to determine canine regional blood flow in ischemic myocardium have shown reduction of regional flow to be proportional to the severity of myocardial damage.\(^{30}\) Therefore, we can conclude that highest antimyosin (Fab')\(_2\) uptake occurred in regions of most severe myocardial damage.

Demonstration of hot spot images in dogs with persistent coronary artery occlusion required a minimum of 48 hr; clear images were obtained at 72 hr. Distinct visualization of localized labeled antibody is dependent on (i) the background radioactivity in circulating blood at the time of gamma imaging, (ii) the rate of entry of the \(^{131}\)I-Ab(Fab')\(_2\) into the infarcted region of the myocardium, and (iii) the use of radioisotopes which are more suitable for gamma scintigraphy than Iodine-131. The half-life of I-(Fab')\(_2\) in the circulation is relatively long.\(^{18}\) An increased rate of concentration of antibody in infarcted myocardium should promote the earlier establishment of a favorable ratio between infarct and blood pool, thereby allowing earlier imaging. Reperfusion of infarcted areas would, of course, permit more rapid equilibration than effected by collateral circulation alone. In animals in which reperfusion occurred 5 hr after occlusion, the ratios of uptake of antibody in infarcted to normal myocardium was 20.7 ± 2.2, as compared to 11.4 ± 0.7 in animals without reperfusion, both measurements being the means of tissue samples with flow rates from 0 to 20% of normal. Improved concentration ratios in the reperfused preparation were correlated with the earlier appearance of distinct hot spots, always possible at 48 hours, whereas in the latter preparation, 72 hours was generally required. At present we are investigating use of other radioisotopes for labeling antimyosin antibody in order to obtain optimal scintigraphic resolution which is not feasible with Iodine-131 employing the conventional Anger camera.

Iodine-131 labeled antimyosin (Fab')\(_2\) fragments can be utilized for specific visualization of myocardial infarcts. Accumulation of the labeled antibody occurred in sufficient concentration for definition of a hot spot by gamma scintigraphy only in tissue samples where regional blood flow has been reduced by greater than 50% of normal. Ratios of antibody uptake are inversely related to regional myocardial blood flow with highest ratios of antibody in myocardial samples of most severe flow compromise.

References

Table 1. Relationship Between Antimyosin (Fab')\(_2\) Uptake, Weight (g) of the Left Ventricular Biopsies and Regional Blood Flow in Infarcted Myocardium

<table>
<thead>
<tr>
<th>Flow</th>
<th>1-10</th>
<th>11-30</th>
<th>31-50</th>
<th>51-80</th>
<th>&gt; 80</th>
</tr>
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<tbody>
<tr>
<td>Persistent occlusion</td>
<td>[Ab]/[Ab]</td>
<td>15.2</td>
<td>11.9 ± 0.5</td>
<td>8.5</td>
<td>7.5 ± 1.1</td>
</tr>
<tr>
<td>W (g)</td>
<td>1.564</td>
<td>10.150</td>
<td>1.080</td>
<td>8.690</td>
<td>7.590</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>[Ab]/[Ab]</td>
<td>20.6 ± 3.5</td>
<td>18.1 ± 1.2</td>
<td>13.0 ± 1.0</td>
<td>2.3 ± 1.6</td>
</tr>
<tr>
<td>W (g)</td>
<td>2.518</td>
<td>4.125</td>
<td>7.610</td>
<td>2.300</td>
<td>1.404</td>
</tr>
</tbody>
</table>

*Mean ± SE.
I = infarct myocardium, N = normal myocardium.
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