RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Imaging of Experimental Myocardial Infarction with Technetium-99m 2,3-Dimercaptosuccinic Acid

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We have studied the use of Tc-99m-labeled 2,3-dimercaptosuccinic acid (Tc-99m DMSA) to scintigraph acute myocardial infarction after coronary occlusion in dogs. Optimal images were obtained 5 hr after injection of radiotracer, with consistent delineation 48 hr after occlusion. Delivery of tracer was dependent on blood flow. Uptake of tracer correlated to extent of infarction as determined by the myocardial depletion of creatine kinase. Myocardial Tc-99m DMSA was protein-bound.

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Imaging of acute myocardial infarction with radiopharmaceuticals has enhanced the ability to diagnose and locate infarction (*1,2*). The most popular agent for this purpose has been Tc-99m pyrophosphate (Tc-99m PPi). This tracer, however, has limitations, which include a delay after the onset of infarction to peak sensitivity, the concentration of Tc-99m PPi in bone, and the nonspecific uptake in patients with conditions other than acute myocardial infarction (*3,4*). In a search for agents likely to avoid some of these drawbacks, we investigated the use of Tc-99m-labeled 2,3-dimercaptosuccinic acid (Tc-99m DMSA) for myocardial imaging.

The study of this radiopharmaceutical is attractive for several reasons.

1. Its safety has been established in routine imaging (*5,6*).

2. It has been postulated that during myocardial injury, damaged protein structures may expose abundantly present sulfhydryl groups for binding (*7,8*). It is conceivable that radiopharmaceuticals such as Tc-99m DMSA could bind to these sulfhydryl groups.

3. Tc-99m DMSA tissue distribution is similar to that of mercury compounds known to bind to infarcted myocardium (*9*).

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In order to explore the use of Tc-99m DMSA in imaging acute infarction, we performed studies in dogs following coronary occlusion. The distribution, disappearance, and binding of Tc-99m DMSA were investigated. In order to determine the dependence of radiotracer binding on blood flow, extent of infarction, and availability of sulfhydryl groups, we measured regional myocardial blood flow, creatine kinase depletion, and sulfhydryl concentration. In addition, the ability to image infarction at various intervals following coronary occlusion with Tc-99m DMSA was determined.

METHODS

Animal model. Twenty-three mongrel dogs, weighing 15–30 kg, were anesthetized with 30 mg/kg of pentobarbital. A left thoracotomy was performed, and the left anterior descending coronary artery dissected free beyond the first medial branch. A silk suture (00) was placed around the vessel and tied. For experiments performed to determine the coronary blood flow with radioactive microspheres, catheters were inserted into the left atrium and carotid artery and exteriorized for later use. After producing coronary occlusion, the animal's chest was closed. To provide controls, in three dogs the coronary artery was isolated but not tied.

Preparation of Tc-99m DMSA. 2,3-DMSA was obtained either in the form of a commercial kit* containing 0.55 mg/ml 2,3-DMSA and 0.21 mg/ml anhydrous

stannous chloride, or as an in-house kit made by our radiopharmacist, containing 0.5 mg/ml of 2,3-DMSA and 0.05 mg/ml stannous chloride. Twenty millicuries of pertechnetate (Tc-99m) were added to the kit, and the contents of the vial thoroughly mixed and allowed to incubate 10 min at room temperature. Before injection, thin-layer silica gel chromatography in saline and acetone was performed on all kits, and those with more than 1% free or 5% hydrolyzed Tc-99m were rejected.

Imaging. Each dog received 20 mCi of Tc-99m DMSA through the jugular catheter, followed by flushing with 20 cc of saline. Blood clearance studies were conducted to determine the optimum time for cardiac imaging. Four groups of three dogs each were imaged at either 12, 24, 48, or 72 hr after coronary occlusion. In vivo images were obtained in the anterior, right and left anterior oblique, and left lateral views. Light anesthesia was maintained with pentobarbital.

The cardiac scintigrams, 500,000 counts per view, were obtained with a 37-PM gamma camera interfaced to a computer. Care was taken to shield out kidney and liver activity. The animals were then killed with an overdose of pentobarbital and the hearts rapidly excised and rinsed in cold saline.

Images of the excised hearts were obtained from the same angles as those in vivo, using the same camera and computer, with 80,000 counts per image. Images were made directly from the analog camera readout, without computer enhancement. Scintigrams were evaluated by three individuals.

In dogs studied 48 hr after coronary occlusion, samples of normal and infarcted myocardium, rib, chest wall, lung, trachea, esophagus, kidney, liver, spleen, and blood were counted in a well counter. Radioactivity was expressed as cpm/g and reported as the ratio of organ radioactivity divided by blood radioactivity in order to normalize data between experiments.

Biochemistry. In the remaining eight dogs, Tc-99m DMSA uptake and creatine kinase depletion (three dogs), regional myocardial blood flow (three dogs), and sulfhydryl concentration (two dogs), were determined 48 hr after coronary occlusion. After 10–14 myocardial samples of normal, border zone, and infarcted areas from each dog were counted in a well counter, the material was prepared for biochemical analysis or for determination of regional blood flow.

Samples for creatine kinase (CK) determinations were homogenized in 10 ml/g of sucrose 0.25 *M*, neutralized EGTA 0.005 *M*, and mercaptoethanol 0.001 *M*, then centrifuged twice at 15,000 rpm for 20 min. The supernatant fraction was assayed for CK activity spectrophotometrically as previously described (*10*). Potential contributions of myokinase to apparent CK activity were excluded by evaluation both with and without creatine phosphate as substrate. Previous investigations have shown that creatine kinase depletion correlates with morphometrical, histological, and physiological estimates of the extent of infarction (*10,11*).

In order to determine whether the binding Tc-99m DMSA depends on sulfhydryl concentration, this and Tc-DMSA activity were determined simultaneously in paired samples obtained from normal and infarcted regions. Myocardial samples for measurement of total, protein-bound, and nonprotein sulfhydryl groups were prepared as previously described (*12*). Sulfhydryl concentration was determined spectrophotometrically by the method of Ellman (*12*). In this reaction, 5,5′-dithio-bis(2-nitrobenzoic acid) is reduced by sulfhydryl to form 1 mole of nitro-(5-mercapto)benzoic acid per mole of sulfhydryl group. In our laboratory, sulfhydryl concentrations in rat liver, muscle, and heart are within 1% of those previously described (*12*). Total protein determination was also performed using Lowry's protein assay (*17*).

Measurement of regional myocardial blood flow. Microspheres labeled with strontium-85 were purchased.† In preparation for injection, the microsphere vials were shaken vigorously with a vortex mixer for at least 15 min. Then 1–5 ml of microsphere suspensions (containing 1–3 million spheres) were injected into the left atrium over 20 sec. Blood was drawn from the carotid artery at a constant rate,‡ beginning 20 sec before injection and continuing for 3 min. Necropsy samples were counted in a two-channel well scintillation counter for Sr-85 and Tc-99m DMSA simultaneously. In experiments using standard solutions of Tc-99m DMSA and the Sr-85 microspheres, there was no appreciable overlap in

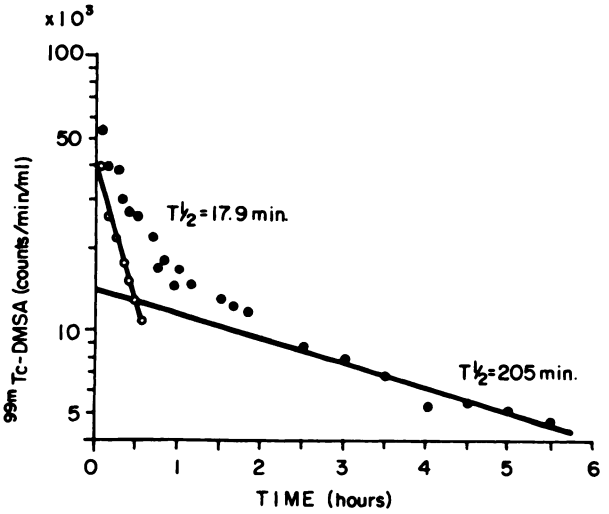

FIG. 1. Plasma clearance of Tc-99m DMSA. Radioactivity from serial plasma samples is plotted on semilog scale. Disappearance is biexponential. Tangent from second portion of curve was extrapolated back to time zero and points on this line subtracted from observed data points (closed circles) to give second set of data points (open circles). These gave half-time of fast phase of clearance. Plasma levels of activity had reached 10% of peak values by 5 hr after injection.

TABLE 1. TECHNETIUM-99m DMSA MYOCARDIAL IMAGING IN DOGS

No. of dogs	Hr after coronary occlusion	Positive images after excision	Positive images in situ
3	12	3	1
3	24	3	3
3	48	3	3
3	72	3	3

pulse-height spectra. Regional blood flow was calculated as previously described (*13*). Correlation coefficients were calculated on a computer. Correlation coefficients between Tc-99m DMSA and creatine kinase, sulfhydryl concentration, and regional blood flow were calculated for each experiment, and results were expressed as the range of correlation coefficients in each group.

RESULTS

Clearance. Measurement of Tc-99m DMSA clearance from the plasma produced a biexponential curve. Half-life of the initial (fast) phase of clearance was 17.2 ± 0.7 min (mean ± s.d.), and for the second (slow) phase was 157 ± 37 min. By 4–5 hr, blood activity had fallen to 10% of the postinjection peak (Fig. 1), and imaging was therefore carried out at this time.

In vivo scintigrams. Dogs that underwent only isolation of the coronary artery did not demonstrate myocardial uptake of Tc-99m DMSA. In animals with coronary occlusion, there was clear demarcation of infarcted tissue 12 hr later in one out of three experiments. From 24–72 hr after coronary occlusion, all animals showed increased Tc-99m DMSA uptake in infarcted areas, with clearest delineation of infarcted tissue occurring at 48 hr (Table 1). The high left thoracotomy incision also took up Tc-99m DMSA, but did not interfere with cardiac activity (Fig. 2). Some animals showed faint rib uptake with the commercial kit, but this did not obscure cardiac images.

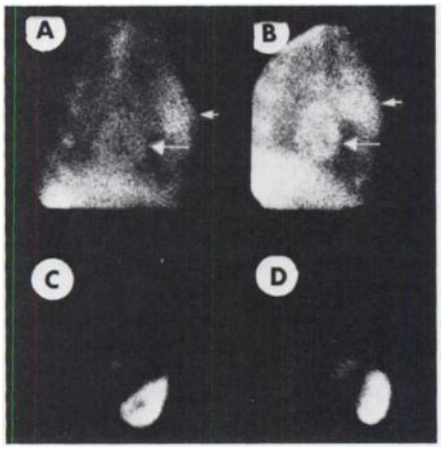

FIG. 2. Myocardial scintigrams with Tc-99m DMSA. Images were obtained in intact dog 48 hr after coronary occlusion and 5 hr after injection of 20 mCi of Tc-99m DMSA. *A* indicates anterior view, *B* indicates left anterior oblique (LAO, 30°). Dense concentration is present in region of myocardial infarct. Short arrow points to operative scar; long arrow to infarcted heart. *C* and *D* are anterior and LAO (30°) views of the excised heart.

In vitro scintigrams. None of the sham-operated dogs showed any localized increase in radioactivity. All isolated hearts from 12–72 hr showed increased Tc-99m DMSA uptake in the area supplied by the ligated coronary artery (Table 1). Computer-generated regions of interest were placed over infarcted and normal myocardium, and the derived count ratios were 2.75 ± 0.4 (s.e.m.) at 12 hr, 2.18 ± 0.37 at 24 hr, 6.23 ± 1.0 at 48 hr, and 2.9 ± 0.3 at 72 hr.

In vitro tissue counts. Infarct-to-normal count ratios from the excised hearts were 4.3 ± 0.62 at 12 hr, 4.8 ± 0.66 at 24 hr, 22.4 ± 3.57 at 48 hr, and 4.9 ± 0.60 at 72 hr. Infarct-to-blood ratios were highest at 48 hr (34 ± 0.3), which corresponded to the time of the highest infarct-to-normal tissue count ratio. The tissue distribution of Tc-99m DMSA was highest in kidney, next in infarcted myocardium, and then in liver (Fig. 3). Liver and kidney are easily shielded and did not interfere with myocardial imaging in this study.

Regional myocardial blood flow. This correlated inversely with Tc-99m DMSA uptake, with a range of correlation coefficients from −0.85 to −0.95 in three dogs (Fig. 4). These data suggest that the delivery of Tc-99m DMSA is related to regional myocardial blood flow.

Extent of myocardial infarction. This was determined by creatine kinase depletion, and was also found to be related to Tc-99m DMSA uptake. With increasing infarction, more CK is released from myocardium, and therefore CK content of the tissue and Tc-99m DMSA correlated inversely, with a range of correlation coefficients from −0.70 to −0.98 in three dogs. In one exper-

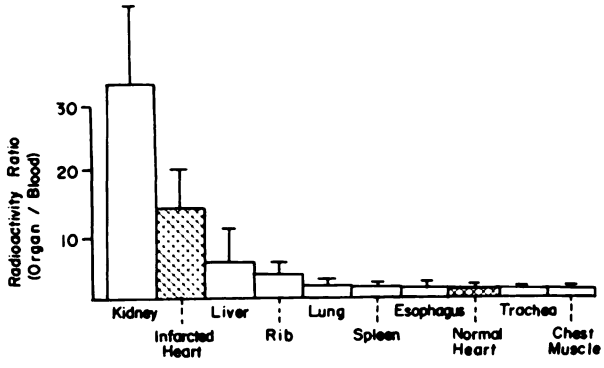

FIG. 3. Distribution of Tc-99m DMSA in dogs with experimental infarction. Bars represent standard error of mean. Myocardial bars are shown hatched. Radioactivity ratios were obtained by dividing activity of organ (cpm/g) by blood (cpm/g).

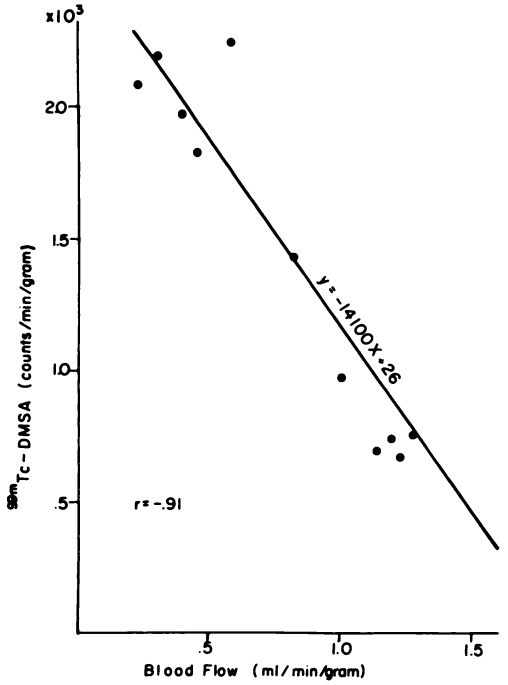

FIG. 4. Regional myocardial blood flow and Tc-99m DMSA concentration in one dog. Delivery of Tc-99m DMSA was closely but inversely correlated with blood flow.

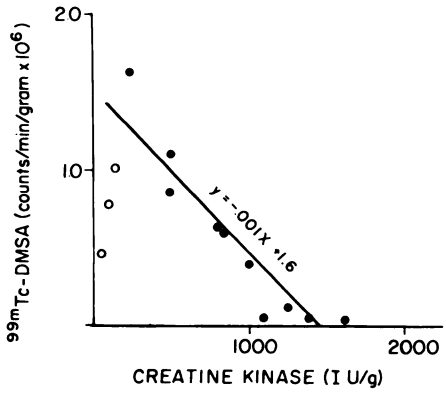

FIG. 5. Extent of infarction and Tc-99m DMSA concentration in one dog with "doughnut" pattern uptake on scintigram. In all dogs, extent of infarction, as determined by depletion of enzyme creatine kinase, was closely related to uptake of Tc-99m DMSA (see text). In this animal, however, with severely infarcted regions and a "doughnut" scintigram, uptake of Tc-99m DMSA was reduced with creatine kinase <200 IU/g. Excluding center biopsies (O), in this experiment, creatine kinase activity correlated inversely with Tc-99m DMSA activity ($r = -0.94$).

iment with a "doughnut" pattern of Tc-99m DMSA uptake, central areas of severe infarction with the greatest release of CK also showed reduced Tc-99m DMSA uptake; these data points were not included when calculating the correlation coefficient for this experiment (Fig. 5).

Sulfhydryl groups. The sulfhydryl concentrations in tissue homogenates from excised infarcted areas were compared with normal areas in two dogs. Total sulfhydryl, protein-bound sulfhydryl and free sulfhydryl levels (millimole/100 g ± s.d.) in infarcted areas (0.46 ± 0.07, 0.43 ± 0.06, 0.03 ± 0.01) were reduced compared with normal areas (0.76 ± 0.08, 0.57 ± 0.06, 0.19 ± 0.02) (all $p < 0.01$). Correction of each sample for protein content had no effect on these reduced sulfhydryl concentrations in infarcted tissue, and thus protein depletion did not account for the reduced levels. Tc-99m DMSA uptake did not correlate with sulfhydryl concentration.

In tissue homogenates prepared for the sulfhydryl assay, protein precipitation resulted in a supernatant containing only (5 ± 3)% (s.d.) of the radioactivity, compared with (95 ± 6)% of the radioactivity in the protein pellet ($p < 0.01$). This is consistent with myocardial protein binding of Tc-99m DMSA.

DISCUSSION

The findings show that acute myocardial infarction in dogs may be imaged successfully with Tc-99m DMSA. Optimal images were not obtained until 48 hr after coronary occlusion, and in this regard Tc-99m DMSA appears no better than Tc-99m PPi in the dog model. This model also showed some skeletal uptake of Tc-99m DMSA which, since it does not occur in man, represents an interesting species variation.

The investigation was initiated by the observation that Tc-99m DMSA behaves much as does Hg-203 chlormerodrin, which was shown to accumulate in acute myocardial infarction (*7,9*). Both tracers are effective renal imaging agents. Chlormerodrin is believed to bind to sulfhydryl-containing proteins in renal cortical tubular cells, partly in the soluble fraction, and partly in mitochondria (*14*). Mercury binding to sulfhydryl groups of proteins from damaged myocardial cells is the mechanism postulated for uptake in acute myocardial infarction.

We did not demonstrate a specific binding mechanism for Tc-99m DMSA or a relationship to sulfhydryl concentration. We found decreased levels of total, free, and protein-bound sulfhydryl groups in infarct tissue, and this would not support Tc-99m DMSA binding to sulfhydryl groups. Most of the radioactivity was protein-bound, which suggests that the mechanism of binding is in part related to myocardial protein binding. Nevertheless, from these studies we cannot totally exclude binding to sulfhydryl groups, since conformational changes of sulfhydryl groups may have occurred, thereby changing the availability of these moieties and accounting for Tc-99m DMSA in vivo binding despite reduced sulfhydryl concentrations.

Delivery of Tc-99m DMSA to the myocardium appears to be similar to delivery of Tc-99m PPi, which is also blood-flow dependent (*15*). Over a broad range, reduced blood flow results in more infarction and greater accumulation of Tc-99m DMSA. Reduced radioactivity in the center of severely infarcted areas in one experi-

ment may be related to insufficient blood flow to deliver tracer. In our laboratory the correlation between CK content of tissue and regional myocardial blood flow ranges between 0.91 and 0.98 (*16*). Therefore, we believe that the reduction of Tc-99m DMSA in the central areas of a "doughnut" pattern on cardiac scintigrams represents failure to deliver tracer. Delivery of Tc-99m DMSA was inversely proportional to regional blood flow at most levels. However, at low levels of flow, failure of delivery may occur, and the relationship between flow and Tc-99m DMSA may become direct. Study of further "doughnut" patterns of uptake would be necessary to define the point where reduced flow impedes delivery. Others have reported a similar dependency on blood flow for delivery of Tc-99m PPi (*15*).

Tc-99m DMSA uptake in infarcted tissue showed good correlation with the degree of enzyme depletion, a marker for the extent of infarction. The presence of some central areas of infarction with reduced blood flow and reduced radioactivity complicates estimates of infarct size with these tracers if estimates do not recognize that the areas with reduced radioactivity are severely infarcted. In this study we did not investigate inferoposterior infarctions. Nevertheless, location of infarction on in vivo scans corresponded closely to the appearance of the infarction on scans of excised hearts. The presence of liver uptake or scar uptake presented no problems with proper shielding and multiple views in the dogs in this study.

The major potential advantage of Tc-99m DMSA over currently used infarct-imaging agents may be the absence of rib uptake in humans. Although rib uptake is used to grade the intensity of Tc-99m PPi uptake, there are times when rib activity obscures activity in an infarct. The infarct-to-normal count ratios obtained in computer-generated regions of interest in this study suggest the need for contrast enhancement if this agent is to be used in patients. The specificity of the agent, compared with Tc-99m PPi, and its potential usefulness in patients remain to be determined. However, comparative studies, and greater characterization of the specific mechanisms responsible for binding of these agents, may lead to specific indications for the use of Tc-99m DMSA for diagnosis and location of acute myocardial infarction.

FOOTNOTE

* M.P.I. DMSA, Medi-Physics, Inc.
† 3M Company.
‡ Harvard Apparatus.

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