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MEASUREMENT OF ACUTE MYOCARDIAL INFARCTS IN DOGS WITH ^{99m}Tc-STANNOUS PYROPHOSPHATE SCINTIGRAMS

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Myocardial scintigrams using ^{99m}Tc-stannous pyrophosphate (^{99m}Tc-PYP) can be used to measure myocardial infarcts produced in dogs by proximal ligation of the left anterior descending coronary artery. Seven dogs had ^{99m}Tc-PYP myocardial scintigraphy performed 24–32 hr after ligation of the proximal left anterior artery. In each dog the scintigrams showed increased ^{99m}Tc-PYP uptake in the distribution of the artery. The scintigraphically visible areas of infarction, measured using interactive computer-aided techniques, were compared subsequently with independent histologic measurements of myocardial infarct size. Several methods for using the area measurements to estimate infarct size were tested. The most successful method ($r = 0.92$, $p < 0.01$) assumed a linear relationship between the largest scintigraphic infarct area and the histologically determined infarct weight. The results suggest that ^{99m}Tc-PYP myocardial scintigrams provide a useful noninvasive method for measuring infarct size in dogs with proximal ligation of the left anterior descending coronary artery.

Cardiac pump failure in patients following acute myocardial infarction has been shown to be directly related to the mass of irreversibly damaged myocardial tissue (*1*). Estimates of infarct size following acute myocardial infarction could, therefore, have important implications regarding prognosis, selection of a treatment regimen, screening patients for surgical revascularization, and evaluating physiologic and pharmacologic therapy for reducing infarct size and preventing extension. Several methods have been used previously to measure infarct size in animals (*2–4*) but none has gained universal acceptance. Bonte, et al have recently shown the utility of ^{99m}Tc-stannous pyrophosphate (^{99m}Tc-PYP) scintigraphy (Mallinckrodt Chemical Works, St. Louis, Mo.) for direct visualization of myocardial infarction in animals and man (*5–7*). The present study was performed to assess the use of ^{99m}Tc-PYP myocardial scintigrams to estimate acute myocardial infarcts in dogs.

METHODS

Adult dogs of either sex, weighing between 15 and 35 kg, were anesthetized with intravenous chloralose (60 mg/kg) and ventilated with a Harvard respirator using 95% O_2 and 5% CO_2. The chest was opened through a median sternotomy and the heart exposed through an incision in the pericardium. The proximal

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FIG. 1. Representative ^{99m}Tc-stannous pyrophosphate myocardial scintigram obtained from dog with acute myocardial infarction. Left-hand panel represents anterior view; middle panel, left anterior oblique view; right panel, left lateral view.

left anterior descending coronary artery was ligated just distal to the first septal branch. The dog's chest was closed and he was allowed to recover for 24–32 hr. This experimental model of myocardial infarction results in an anterior myocardial infarction and a positive ^{99m}Tc-PYP myocardial scintigram with the radionuclide uptake in the histologic area of damage (*8*). Imaging was carried out 1 hr after an intravenous injection of 3 mCi (5 mg) of ^{99m}Tc-PYP. Scintigrams were made using a Searle Radiographics Pho/Gamma III HP camera with a high-resolution collimator. At least three different views (anterior, left anterior oblique, and lateral) were obtained in each animal (Fig. 1). Data were collected by a PDP-8/I computer interfaced to the scintillation camera and were recorded on seven-track magnetic tape as a 64 × 64 matrix for later off-line processing. Each static image contained at least 300,000 counts.

Each digital image was retrieved from tape and placed in computer memory for processing. Golay edge detection was then applied to each scintigram (*9*). The Golay technique is a method for automatically locating boundaries in an image. Because the method is consistent in determining boundaries from image to image, it produces infarct areas with less subjective error than manual techniques.

Histologic quantitation of infarct size was performed by utilizing the methods of Alonso, et al (*10*) and Reimer, et al (*11*). Immediately after the scintigraphic study, the dogs were killed and the hearts removed. The hearts were divided into five or six transverse slices after the brief coronary perfusion through the aortic roots with 10% phosphate-buffered formalin. Each formalin-fixed heart was weighed. The right ventricular free wall, atria, and extraneous connective tissue were dissected from the ventricular slices leaving a mass of myocardium consisting of interventricular septum and left ventricular free wall, considered in this study to represent the mass of the left ventricle. Each slice of left ventricular myocardium was divided into several blocks,

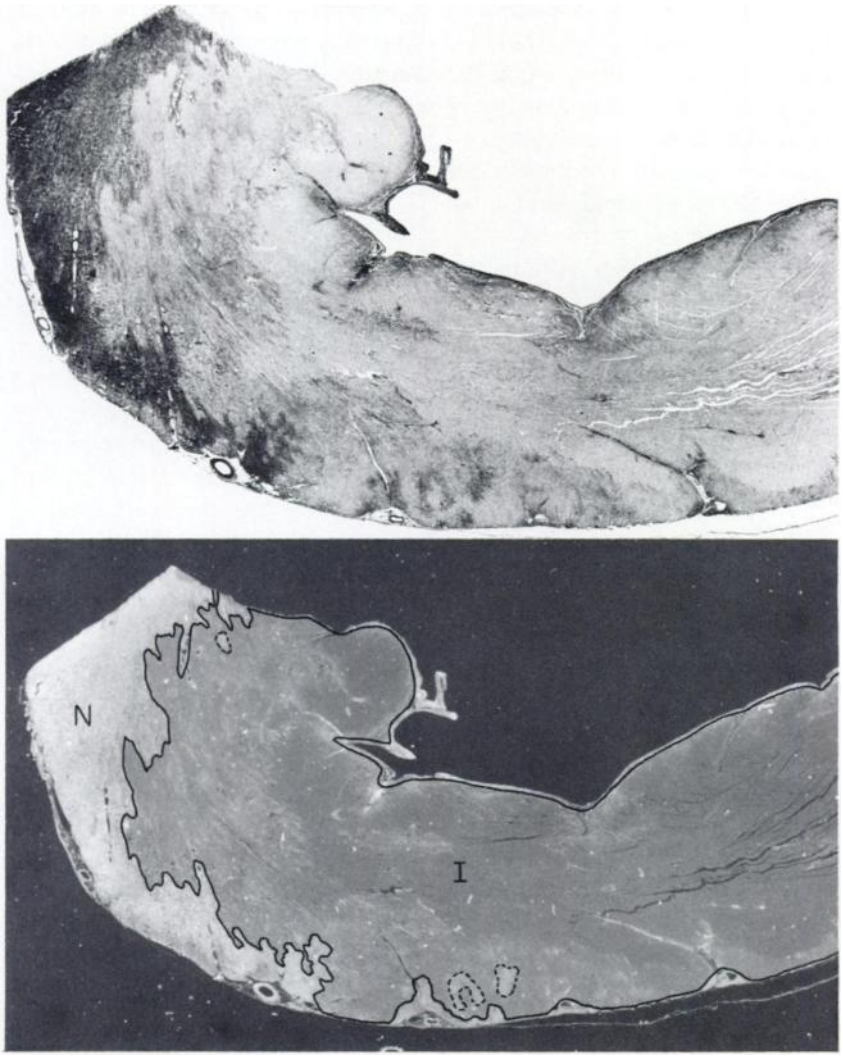

FIG. 2. Positive (top) and negative (bottom) photographs of PAS-stained section prepared from block of left ventricular myocardium from dog subjected to arterial ligation for 1 day. Non-necrotic myocardium (N) is intensely stained due to abundant glycogen deposits. Infarcted myocardium (I) exhibits variable staining due to differences in neutrophil content and in intensity of diffuse diastase-resistant PAS staining of necrotic muscle cells. On negative photograph, infarcted area is enclosed within solid line, and few foci of non-necrotic myocardium located in infarct border are enclosed within dotted lines. Planimetry of negative photograph is used to determine total section area and area of infarcted myocardium (area within solid line minus areas within dotted lines). Ratio of infarct area to total area is calculated and multiplied by weight of block to estimate mass of infarcted myocardium in block. (PAS stain; top × 7.2, bottom × 6.5).

usually four or five, and each block was weighed. The histologic sections were cut to 6–8 μm thickness and stained with hematoxylin and eosin or with the periodic acid-Schiff (PAS) technique, some with and others without prior diastase digestion. The sections stained with PAS without prior diastase digestion were placed in a photographic enlarger, and negative photographic prints of the sections were prepared at a standard magnification. Areas of infarcted myocardium were traced onto the photographic prints (Fig. 2). For each section, the total area and the area of infarcted myocardium were obtained by planimetry, and the percent of infarcted myocardial area in each section was calculated. The mass of infarcted myocardium in each block was calculated by multiplying the fraction of infarcted myocardium by the weight of the block. From these calculations the following data were obtained: (A) total mass of infarcted myocardium; (B) percent of left ventricular mass infarcted; and (C) percent of total heart weight occupied by infarcted myocardium.

Correlations were made between infarct area, measured using the laboratory computer system and the Golay outline method, and the morphologic infarct weight. Four methods were used to treat the data. The following quantities were plotted and subjected to calculations of linear regression, correlation coefficients, residual root mean square error, and p values for testing statistical significance: (A) infarct area as a percent of the "radioactive blood pool" area versus infarct weight as a percent of the whole heart weight; (B) infarct area as a percent of the "radioactive blood pool" area versus infarct weight; (C) infarct area versus infarct weight; and (D) the best (least squares sense) linear combination of the three areas for all seven animals versus infarct weight.

TABLE 1. QUANTITATIVE MORPHOLOGIC DATA IN SEVEN DOGS WITH PROXIMAL OCCLUSIONS OF THE LEFT ANTERIOR DESCENDING CORONARY ARTERY

Dog	Heart weight (gm)	Left ventricular weight (gm)	Weight of infarct (gm)	Percent of left ventricle infarcted	Percent of heart infarcted
E	148	99.6	21.4	21.5	14.5
J	73.5	44.5	10.7*	24.1	14.6
K	124	75.4	22.7*	30.0	18.3
P	73.5	41.8	9.2*	21.9	12.5
R	126	77.8	26.9	34.6	21.3
S	113	71.0	11.5	16.2	10.2
U	99	55.1	3.0	5.4	3.0

* Infarct weights were determined by averaging results obtained from histologic sections taken from apical and basal surfaces of most tissue blocks.

A correlation coefficient was calculated for the linear regression line and the data points. Probability values less than 0.05 were required for significant rejection of the null hypothesis.

RESULTS

The quantitative pathologic data are presented in Table 1. In most histologic sections, areas of infarction were relatively homogeneous and contained relatively small border zones with mixtures of necrotic and non-necrotic myocardium. In the PAS-

TABLE 2. SCINTIGRAPHIC MEASUREMENTS OF INFARCT SIZE (N = 7)

		Left anterior oblique			Anterior		
		r	p value	rrms*	r	p value	rrms*
Infarct area/blood-pool area versus infarct weight/whole-heart weight	60% Golay threshold†	0.552	not sig.	24.4	0.656	not sig.	36.1
	75% Golay threshold	0.539	not sig.	31.6	0.407	not sig.	27.1
	90% Golay threshold	0.591	not sig.	42.1	0.391	not sig.	53.7
Infarct area/blood-pool area versus infarct weight	60% Golay threshold	0.607	not sig.	23.3	0.74	not sig.	22.1
	75% Golay threshold	0.628	not sig.	29.2	0.486	not sig.	25.9
	90% Golay threshold	0.675	not sig.	38.5	0.431	not sig.	52.6
Infarct area versus infarct weight	60% Golay threshold	0.628	not sig.	8.10	0.814	<0.05	7.4
	75% Golay threshold	0.832	<0.02	5.0	0.792	<0.05	3.9
	90% Golay threshold	0.865	<0.01	2.6	0.642	not sig.	4.1
Best linear combination of three areas versus infarct weight	60% Golay threshold	0.905	<0.01	4.0 (gm^2)			
	75% Golay threshold	0.834	<0.02	5.3 (gm^2)			
	90% Golay threshold	0.887	<0.01	5.5 (gm^2)			
Largest area versus infarct weight	Manual method	0.92	<0.01	3.6			
	75% Golay threshold	0.857	<0.01	4.0			

* Residual root mean square error (cm^2).

† The Golay threshold represents a percent of the maximum activity (counts) in the area of infarction. The boundary of the infarct is selected by the Golay algorithm where the activity surrounding the infarct falls off to the level of the Golay threshold.

stained sections, differences in intensity of staining were accounted for on the basis of (A) abundant intensely PAS-positive glycogen deposits in nonnecrotic myocardium, (B) glycogen depletion and diffuse diastase-resistant PAS staining of necrotic muscle cells in the infarcts, and (C) PAS staining of neutrophils in the infarcts. The generally more intense staining of the peripheral as compared to the central zones of the infarcts was due to a combination of (A) more intense diastase-resistant PAS staining of muscle cells in the peripheral areas and (B) selective infiltration of these areas by neutrophils. The areas of infarction on the negative photographs were outlined only after those areas were found to have the classic histopathologic features of necrosis upon examination of the hematoxylin-and-eosin-stained sections corresponding to those stained with PAS.

Table 2 presents the correlation coefficients, p values, and residual root mean square errors for several methods for estimating infarct weight from projected infarct area. Results are shown for 90, 75, and 60% Golay thresholds. Only anterior and left anterior oblique data are presented since areas measured from the left lateral projections proved to be poor estimators of infarct weight.

Figure 3 shows a plot of the largest scintigraphic projections of infarct area versus morphologic infarct weights using areas measured manually. That scintigraphic view with the largest measured infarct area was chosen for this comparison. In all cases this was either the anterior or left anterior oblique view. The results are also representative of those obtained with the Golay algorithm since there was no significant difference between scintigraphic areas obtained utilizing either method (Table 2).

DISCUSSION

The results of this study show that infarct size can be adequately measured with ^{99m}Tc-PYP myocardial scintigrams in dogs with proximal left anterior arterial ligation. The best correlation between scintigraphic and histologic measurements of infarct size was obtained when the largest scintigraphic infarct area from one of the three views was plotted against morphologic infarct weight (Fig. 3). We did not find a good correlation between scintigraphic infarct area and infarct weight expressed as a percent of total heart area. This is probably due to the imprecise definition of the cardiac silhouette when a ^{99m}Tc-PYP scintigram is used to outline the entire heart (Table 2).

The infarcts produced in these animals, using ligation of the proximal left anterior descending coronary artery, were all anterior in location. While animal infarct models with septal, true posterior, and other locations may show different relationships to infarct weight, these results suggest that a simple measurement of infarct area from the ^{99m}Tc-PYP scintigram provides a good estimate of infarct weight for anterior and anterolateral infarction.

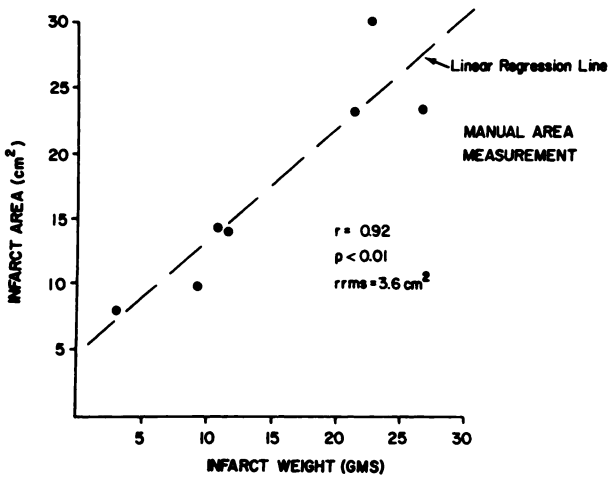

FIG. 3. Relationship between morphologic infarct weight and largest scintigraphic infarct area measured manually.

The potential application of this approach to measuring infarct size in man is currently uncertain, but the data provide sufficient encouragement for this myocardial-imaging technique to be evaluated clinically. Additionally, comparative measurements of infarct size in experimental animals and patients utilizing precordial mapping (*2*) and creatine phosphokinase clearance measurements (*4*) should be made.

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