jnm/ PRELIMINARY NOTE

Double-Labeling of Experimental Acute Myocardial Infarcts with ^{113m}In- and ^{99m}Tc-EDTMP

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The feasibility of double-labeling of acute myocardial infarcts with ^{113m}In-EDTMP [ethylenediaminetetra(methylene phosphonic acid)] and ^{99m}Tc-EDTMP was evaluated. The in vitro distributions of these tracers in acute myocardial infarcts in dogs and their selectivities for infarcted versus non-infarcted myocardium were compared. Both tracers concentrated in acutely infarcted myocardium, and there was excellent correlation between their uptakes ($r = 0.88$). They also provided complete separation between infarcted and uninfarcted tissue, as checked by histology. Accordingly, these agents show promise for the multiple-labeling of acute myocardial infarcts in experiments to determine the natural course of myocardial infarction and the efficacy of therapy aimed at limiting infarct size. In addition, ^{113m}In-EDTMP may be useful for serial scintigraphy during the early phase of acute myocardial infarction when the damage may be, at least to some extent, reversible.

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Many experimental interventions to limit infarct size have been described (*1*). The various techniques currently available for determining the extent of myocardial damage have several disadvantages (*2–4*). Although several ^{99m}Tc-labeled complexes are potentially useful for detecting acute myocardial infarction (*5–7*) and for estimating infarct size (*8–11*), these radiopharmaceuticals may not be suitable for serial relative determinations of infarct size in man during the early stages of acute myocardial infarction because the 6-hr half-life of ^{99m}Tc is long relative to the period during which ischemic changes might remain reversible and therefore amenable to treatment.

We examined the double-labeling of infarct-seeking tracers for the sequential evaluation of myocardial necrosis in experimental acute infarction. The chelating agent, ethylenediaminetetra(methylene phosphonic acid) (EDTMP), was labeled with ^{113m}In and ^{99m}Tc, and its in vitro biologic distribution was determined in dogs sustaining acute myocardial infarction. The correlation between ^{113m}In- and ^{99m}Tc-EDTMP concentrations in normal and infarcted myocardium was determined, as was the selectivity of this technique for infarcted versus non-infarcted myocardium.

METHODS

In its free acid form, EDTMP (Monsanto Co., St. Louis, Mo.) is a white crystalline powder. A stock solution of sodium salt was prepared by titrating the acid to pH 6.5 with NaOH to a final concentration of 40 mg/ml of the acid. The EDTMP was either labeled with ^{99m}Tc using the $SnCl_2$ reduction technique (*12*) or with ^{113m}In by adding the required activity from a ^{113}Sn–^{113m}In generator elution (in 0.05 *M* HCl) to 0.5 mg of the sodium salt. Sodium carbonate buffer was added to bring the final pH to 7.5 (*13*).

Six mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg), intubated endotrachially, and artificially respirated with a Harvard pump. A left thoracotomy in the fifth intercostal space was

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performed, and the heart was suspended in a pericardial cradle. The left anterior descending artery was ligated at its midportion. The chest was closed and the dog was allowed to recover for 23 hr. Then, 3 mCi of ^{113m}In-EDTMP and 1 mCi of ^{99m}Tc-EDTMP were injected intravenously. One hour later, the animals were reanesthetized and reintubated, their chests reopened, and the hearts excised. Transmural specimens (1–2 gm) were obtained from well-perfused and infarcted myocardium, and from border regions. Each specimen was divided longitudinally into two parts, one for histopathologic examination and one for activity counting with a NaI(Tl) scintillation detector. Liver and bone specimens were also obtained.

Sections for histologic examination were fixed in Bouin's solution and stained with hematoxylin and eosin. Standard criteria were used for the histologic diagnosis of acute myocardial infarction. For each tracer, concentration ratios (CR) were determined by the equation

$$CR = \frac{\text{counting rate/gm of tissue specimen}}{\text{counting rate/gm of normal myocardium}}.$$

The tissue specimens could be either frankly infarcted myocardium or tissue adjacent to the infarct, and normal myocardium was tissue perfused by the circumflex artery. Similar ratios were determined for infarcted myocardium relative to liver and bone.

Blood clearances were determined in three dogs for each of the two labeled compounds. Blood samples were obtained at 2, 5, 10, 30, 60, 120, and 240 min after intravenous injection. The percent injected dose remaining in the blood at any time was expressed as the activity in a blood sample divided by the total activity injected. We used the standard assumption that 6.5% of the animal's weight represents blood pool.

RESULTS

When injected 23 hr after coronary occlusion, both ^{113m}In- and ^{99m}Tc-EDTMP concentrated in the acutely infarcted myocardium. The infarct-to-normal-myocardium concentration ratios for these two tracers fell into the following relationship:

$$CR_{In} = 0.98CR_{Tc} + 0.65.$$

The correlation coefficient r was 0.88, and the result was highly significant ($p < 0.001$). The 95% confidence limits are shown in Fig. 1.

Histologically infarcted and uninfarcted tissue could be completely separated by this technique (Fig. 1). All specimens with histologic evidence of infarction had concentration ratios above 4.0 for ^{113m}In-EDTMP and above 3.5 for ^{99m}Tc-EDTMP.

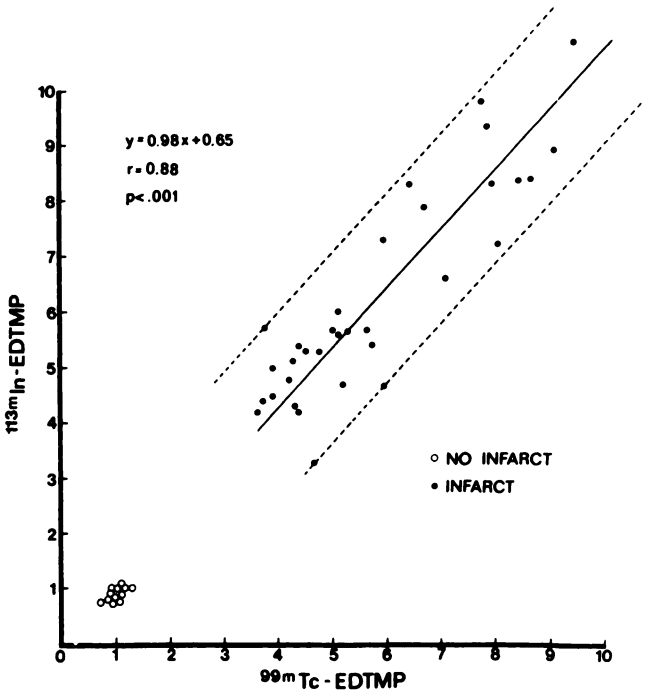

FIG. 1. Correlation between concentration ratios for ^{113m}In-EDTMP and ^{99m}Tc-EDTMP. Regression line is calculated for infarcted tissue only. Dotted lines represent 95% confidence limits. Closed circles denote concentration ratios for infarcted myocardium, and open circles denote concentration ratios for uninfarcted myocardium adjacent to infarct.

The mean concentration ratio was 6.4 ± 0.4 (s.e.m.) for ^{113m}In-EDTMP and 5.9 ± 0.3 for ^{99m}Tc-EDTMP. The percent of injected dose per gram of infarcted myocardium was 0.0081 ± 0.0007 and 0.0051 ± 0.0005 for ^{113m}In- and ^{99m}Tc-EDTMP, respectively. The mean concentration ratios between tissue bordering the infarct (presumably ischemic tissue) and normal myocardium distant from the infarct were 0.91 ± 0.02 for ^{113m}In-EDTMP and 1.00 ± 0.02 for ^{99m}Tc-EDTMP.

The uptake by bone was high, with infarct-to-bone concentration ratios of 0.8 ± 0.4 for ^{113m}In-EDTMP and 0.7 ± 0.2 for ^{99m}Tc-EDTMP. The infarct-to-liver ratios were 4.6 ± 1.2 and 1.9 ± 0.2 for ^{113m}In- and ^{99m}Tc-EDTMP, respectively.

Both tracers cleared rapidly from the blood, with only 6% remaining 1 hr after injection (Fig. 2). After this point, ^{99m}Tc-EDTMP cleared more rapidly than ^{113m}In-EDTMP. Perhaps the slower clearance of ^{113m}In-EDTMP is due to intravascular binding or to ^{113m}In binding with transferrin.

DISCUSSION

The radiopharmaceuticals described in this preliminary report can be used for multiple-labeling of acute myocardial infarcts. The carrier ligand chosen for this study, ethylenediaminetetra(methylene phos-

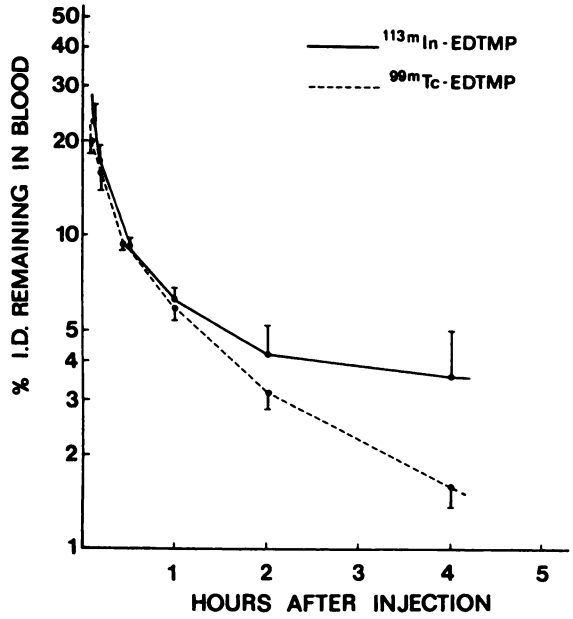

FIG. 2. Blood clearance curves for ^{113m}In-EDTMP and ^{99m}Tc-EDTMP. Percent of injected dose (%ID) remaining in total blood volume was determined from counting rate per gram of whole blood. Error lines indicate standard error of mean.

phonic acid), or EDTMP, is an analog of ethylenediaminetetraacetic acid (EDTA).

In this study, both ^{113m}In- and ^{99m}Tc-EDTMP concentrations were elevated in histologically documented infarcted myocardium only. Furthermore, all portions of the infarct had elevated concentration ratios. This technique is therefore both sensitive and specific in defining the acutely infarcted regions of the myocardium. The infarct-to-normal myocardial concentration ratios obtained with labeled EDTMP are one-half to one-third of those found with such bone-seeking tracers as ^{99m}Tc-pyrophosphate and ^{99m}Tc-HEDP (*6,15*) and approximately the same as the ratios found for ^{99m}Tc-tetracycline (*15*). The percent of injected dose per gram of infarcted myocardium is in the same range as that for ^{99m}Tc-HEDP. Thus, the amount of labeled EDTMP retained by normal myocardium is higher than for ^{99m}Tc-pyrophosphate or ^{99m}Tc-HEDP. The infarct-to-bone ratio is approximately the same as with ^{99m}Tc-HEDP.

The tissue distribution of EDTMP labeled with ^{99m}Tc and ^{113m}In indicates that double-labeling of acute myocardial infarcts is feasible. This would be of value in both animal studies and in man. No technique currently available permits the quantitative determination of changes in the size of the infarcted tissue during that phase when ischemic changes may still be reversible. Double-labeling with EDTMP would permit the experimental investigator to determine changes in infarct size after interventions that either reduce or increase the extent of infarction. The size of the infarct might then be determined either by in vitro counting of tissue specimens or by serial external imaging.

In man, serial studies with ^{99m}Tc complexes that concentrate in infarcted myocardium are hampered by the relatively long half-life of ^{99m}Tc (6 hr). Double-labeling techniques and serial scintigraphy with ^{113m}In-EDTMP, with its 100-min physical half-life, are promising methods to study the natural evolution of infarcts and the efficacy of interventions aimed at limiting infarct size.

This study suggests the feasibility of the double-labeling technique. Although there may be limitations on the use of ^{99m}Tc- and ^{113m}In-EDTMP as tracers for this purpose, radiopharmaceuticals with better physical and biologic properties may indeed be synthesized. The uptake of labeled EDTMP in bone, the lower infarct-to-normal-myocardium concentration ratios compared to other bone agents, and the high photon energy of ^{113m}In, for example, might limit the accuracy of infarct sizing by external imaging. These considerations would not affect in vitro studies. It would be necessary, however, to determine the length of time required after the first injection so that an intervention no longer affected the distribution of the particular tracer within the myocardium.

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