jnm/DIAGNOSTIC NUCLEAR MEDICINE

# SELECTIVE UPTAKE OF $^{99m}$Tc COMPLEXES AND $^{67}$Ga IN ACUTELY INFARCTED MYOCARDIUM

F. G. Zweiman, B. L. Holman, A. O'Keefe, and J. Idoine

*Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts*

***The suitability of various radiopharmaceuticals ($^{99m}$Tc-tetracycline analogs, $^{99m}$Tc-glucoheptonate, $^{99m}$Tc-diphosphonate, and $^{67}$Ga-citrate) for use in imaging acute myocardial infarction was assessed by determining their biologic distributions in experimentally infarcted dogs. The highest infarct-to-normal myocardial concentration ratio was found with $^{99m}$Tc-diphosphonate (27.9:1); the highest infarct-to-liver ratio was also found with $^{99m}$Tc-diphosphonate (15.9:1). The infarct-to-bone ratio, however, was 0.4:1 with $^{99m}$Tc-diphosphonate. There was an excellent positive linear correlation between bloodflow reduction and uptake of the radiopharmaceutical after a threshold reduction in blood flow with $^{99m}$Tc-glucoheptonate and $^{99m}$Tc-tetracycline. Technetium-99m-tetracycline accumulated only in infarcted tissue while $^{99m}$Tc-diphosphonate was increased in both ischemic and infarcted tissue. Thus, $^{99m}$Tc-diphosphonate has characteristics best suited for scintigraphic imaging. Since $^{99m}$Tc-tetracycline accumulates only in infarcted tissue, however, this tracer more accurately defines the size of an acute infarction.***

Recently, scintigraphic techniques have been developed for detecting acute myocardial damage using tracers that accumulate within the damaged tissue (*1–5*). The potential clinical utility of the various radiotracers that have been proposed for acute myocardial scintigraphy depends largely on their biologic properties. First, the specificity of the agent for nonviable infarcted myocardium relative to ischemic tissue, normal myocardium, and surrounding organs must be determined. Second, the clearance rate of the agent from the blood is required, not only to provide a suitable contrast between the lesion and background but also to permit serial scanning. The latter is necessary in order to follow the infarct's boundary, since the viable border zone may become irreversibly damaged within a short time after the initial injury, perhaps within 8 hr (*6*). Alternatively, if agents with different biologic characteristics could be identified, with one tracer labeling infarcted zones only and another labeling both ischemia and infarction, the combined application of these agents would provide information not only on the extent of infarction but on the size of the ischemic zones as well.

We studied a number of $^{99m}$Tc complexes ($^{99m}$Tc-glucoheptonate, $^{99m}$Tc-diphosphonate, and $^{99m}$Tc-tetracycline and its analogs) and $^{67}$Ga-citrate in an animal model to determine (A) the relative concentration of tracer in infarcted and normal myocardium, (B) the blood clearance of the tracer, (C) the concentration in surrounding organs relative to that in the infarct, (D) the quantitative relation between bloodflow reduction and infarct concentration, and (E) the concentration in ischemic relative to infarcted myocardium.

## METHOD

Technetium-99m-tetracycline, $^{99m}$Tc-oxytetracycline, $^{99m}$Tc-dichlorotetracycline, and $^{99m}$Tc-chlorotetracycline were prepared by New England Nuclear Corporation. Commercial preparations of glucoheptonate (NEN), diphosphonate (Procter & Gamble), and $^{67}$Ga-citrate were also obtained. There were 10–20 mg of the tetracycline analog, 100–200 mg of glucoheptonate, or 3–6 mg of diphosphonate in the final dose administered to each animal.

Thirty mongrel dogs (weighing 15–22 kg) were anesthetized with phenobarbital (30 mg/kg). A right carotid artery cutdown was performed. A catheter guidewire system, with a 5-mm piece of occluded catheter material placed on the tip of the guidewire, was introduced into the right carotid ar-

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For reprints contact: B. Leonard Holman, Dept. of Radiology, Harvard Medical School, 25 Shattuck St., Boston, Mass. 02115.

**TABLE 1. CONCENTRATION RATIOS BETWEEN INFARCT AND SURROUNDING TISSUES (± s.e.m.)**

| | Percent injected dose × 1000 / gm infarcted tissue | Infarct:normal | Infarct:liver | Infarct:bone | Infarct:blood | Number of animals |
|---|---|---|---|---|---|---|
| $^{99m}$Tc-diphosphonate | 5.9 ± 1.9 | 27.9 ± 6.5 | 15.9 ± 9.2 | 0.4 ± 0.1 | 68.6 ± 38.9 | 6 |
| $^{99m}$Tc-glucoheptonate | 3.6 ± 1.2 | 11.2 ± 2.7 | 0.7 ± 0.1 | 9.5 ± 1.1 | 5.4 ± 1.3 | 4 |
| $^{99m}$Tc-oxytetracycline | 3.4 ± 0.2 | 9.2 ± 1.6 | 0.6 ± 0.1 | 6.1 ± 0.9 | 4.4 ± 1.6 | 3 |
| $^{99m}$Tc-tetracycline | 4.3 ± 0.3 | 7.7 ± 1.5 | 0.6 ± 0.1 | 15.6 ± 2.1 | 4.5 ± 1.3 | 6 |
| $^{99m}$Tc-chlorotetracycline | 3.2 ± 0.8 | 7.7 ± 0.7 | 0.8 ± 0.2 | 11.5 ± 1.7 | 3.2 ± 0.6 | 3 |
| $^{99m}$Tc-chlorodimethyl tetracycline | 2.7 ± 0.5 | 6.9 ± 1.1 | 0.6 ± 0.1 | — | 3.2 ± 1.2 | 3 |
| $^{67}$Ga-citrate | — | 1.9 ± 0.6 | 0.3 ± 0.1 | — | 3.3 ± 0.8 | 5 |

tery. Under fluoroscopic control the left coronary ostium was selectively entered. After the system was positioned in the main left coronary artery, the guidewire was pulled back, releasing the 5-mm plug into the artery.

At the time of catheterization, 60 μCi/kg of $^{67}$Ga-citrate was given intravenously in five dogs. In the other 25 dogs, 15–20 mCi of a $^{99m}$Tc complex was injected intravenously 24 hr after embolization.

Relative myocardial blood flow was determined by injecting 600,000 $^{85}$Sr-labeled microspheres 15 ± 5 microns in diameter slowly over 20 sec into the left ventricle via a catheter positioned near the apex of the ventricular cavity. We used the intraventricular rather than the intra-atrial route since we have shown a mean difference of 6.5% for each tissue section (left ventricular wall and septum only) when a ventricular injection is compared to an atrial injection. This compares favorably with a mean difference of 4.5% under comparable conditions but with sequential atrial injections.

The animals were sacrificed by injecting concentrated potassium chloride solution intravenously 48 hr after experimental embolization. Representative samples of the liver, lung, blood, and posterior rib were taken. The boundaries of the infarct were determined by gross inspection, and representative sections of infarcted, border zone, and normal tissue were obtained for histologic examination and/or gamma counting. The hearts were divided into 1–2-gm sections that were mapped according to chamber position and relationship to the coronary vessels and plug (7). The radioactivity in tissue samples was measured in a gamma well counter.

After several days, radioactivity in the samples was measured again to determine the concentration of microspheres. The microsphere activity per gram of myocardium was used as an index of blood flow. Only tissue from the septum and left ventricle was analyzed. The tissue sample with the highest microsphere activity was arbitrarily considered 0% reduction in flow. The percent reduction in flow for all other samples was determined by calculating the percent reduction in microsphere activity relative to the sample with the highest concentration. Similar calculations were performed to determine the percent of radiopharmaceutical concentration relative to the sample with the highest concentration of $^{99m}$Tc complex or $^{67}$Ga.

Light microscopic examination of the histologic sections following hematoxylin and eosin staining was performed to determine the presence of myocardial infarction (*8*). In order to evaluate myocardial ischemia in two dogs after injection of $^{99m}$Tc-tetracycline and in two dogs after injection of $^{99m}$Tc-diphosphonate special staining methods were used (hematoxylin–basic fuchsin–picric acid). Technetium-99m-glucoheptonate was not tested at this time because clinical trials were disappointing. Only 3 of 13 patients with acute myocardial infarcts had abnormal scintiscans after the intravenous injection of $^{99m}$Tc-glucoheptonate. Ischemia was diagnosed in a tissue specimen when there was positive fuchsin staining without evidence of infarction on the hematoxylin–eosin section based on standard criteria for the diagnosis of infarction (*8,9*).

To compare blood levels of $^{99m}$Tc-tetracycline, $^{99m}$Tc-oxytetracycline, $^{99m}$Tc-diphosphonate, and $^{99m}$Tc-glucoheptonate at various times after injection, three mongrel dogs were injected with each of the radiopharmaceuticals (4–8 mCi). Blood samples were taken at 3 min, 2–3 hr, 4–5 hr, and 24 hr. The extraction rate of the radionuclides from the blood was calculated as the reduction in radiotracer concentration from the 3-min blood sample.

## RESULTS

Table 1 shows the uptake of the various tracers in the infarcted region, expressed as a percentage of the injected dose and measured 48 hr after the experimental occlusion described. It also shows the concentration ratios between the infarct and other tissues of interest at 48 hr. The gallium-citrate was

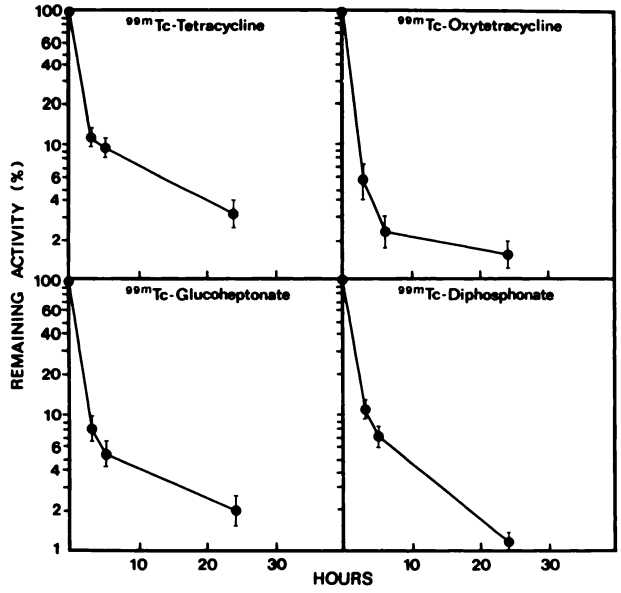


**FIG. 1.** Blood clearance of various $^{99m}$Tc complexes as percent of remaining activity (± s.e.m.).

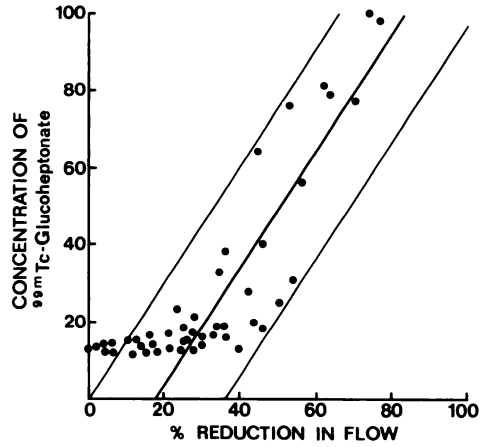


**FIG. 2.** Correlation between percent bloodflow reduction and uptake of $^{99m}$Tc-glucoheptonate in Dog 14. Regression line (middle line) was fitted from threshold bloodflow reduction value to maximum bloodflow reduction ($r = 0.889$). Lines bordering regression line are 95% confidence limits.

administered at the time of the occlusion; the technetium-labeled complexes were given 24 hr later.

The greatest concentration ratio between infarcted and normal myocardium occurred with $^{99m}$Tc-diphosphonate (27.9:1). Technetium-99m-glucoheptonate and $^{99m}$Tc-oxytetracycline resulted in ratios approximately one-third those of the bone agent, but higher than those found with the other tetracycline analogs. Ratios found for $^{67}$Ga-citrate were the lowest.

While the concentration ratio between infarcted and normal myocardium obtained with $^{99m}$Tc-diphosphonate was greater than the other $^{99m}$Tc complexes ($p < 0.025$), there was no significant difference between the concentration ratios of the $^{99m}$Tc-tetracycline analogs with each other and with $^{99m}$Tc-glucoheptonate.

The percent injected dose of $^{99m}$Tc-diphosphonate was 1.7 times greater than $^{99m}$Tc-glucoheptonate and 2.1 times greater than $^{99m}$Tc-oxytetracycline. Consequently, the difference in the concentration ratios between normal and infarcted myocardium for $^{99m}$Tc-diphosphonate and the other $^{99m}$Tc complexes was due both to a greater concentration of the bone agent in infarcted tissue and a decreased concentration in normal myocardium.

The concentration ratio between infarct and liver was approximately ten times greater with $^{99m}$Tc-diphosphonate than with the other $^{99m}$Tc complexes. However, the infarct-to-bone concentration ratio was 0.4:1 with $^{99m}$Tc-diphosphonate while it was between 7.0:1 and 11.5:1 for the other $^{99m}$Tc complexes.

The initial blood washout was most rapid for $^{99m}$Tc-oxytetracycline and $^{99m}$Tc-glucoheptonate, with only 5.9 ± 2.5% and 8.0 ± 1.5% (s.e.m.) of the injected dose remaining at 3 hr, respectively (Fig. 1). The initial washout was slower for $^{99m}$Tc-tetracycline and $^{99m}$Tc-diphosphonate (11.6 ± 1.3% and 11.1 ± 1.1% remaining 3 hr after injection).

Once a threshold level of bloodflow reduction had occurred, positive correlation between the degree of

**TABLE 2. CORRELATION BETWEEN BLOOD FLOW AND CONCENTRATION OF RADIOTRACER**

| Dog number | Number of tissue sections | Threshold* | Regression coefficient† |
|---|---|---|---|
| $^{99m}$Tc-diphosphonate | | | |
| 1 | 25 | 11% | 0.440 |
| 2 | 18 | 15% | 0.897 |
| 3 | 54 | 9% | 0.511 |
| 4 | 44 | 15% | 0.852 |
| 5 | 30 | 6% | 0.822 |
| 6 | 56 | 12% | 0.795 |
| | | 11 ± 2% | |
| $^{99m}$Tc-tetracycline | | | |
| 7 | 15 | 38% | 0.810 |
| 8 | 8 | 60% | 0.908 |
| 9 | 18 | 30% | 0.840 |
| 10 | 26 | 47% | 0.902 |
| 11 | 26 | 52% | 0.865 |
| 12 | 44 | 30% | 0.852 |
| | | 43 ± 4% | |
| $^{99m}$Tc-glucoheptonate | | | |
| 13 | 17 | 26% | 0.921 |
| 14 | 33 | 28% | 0.889 |
| 15 | 16 | 46% | 0.976 |
| 16 | 20 | 55% | 0.963 |
| | | 39 ± 7% | |

* Percent reduction in flow at which concentration of radiotracer begins to increase.

† For regression line fitted from threshold to maximum radiotracer concentration.

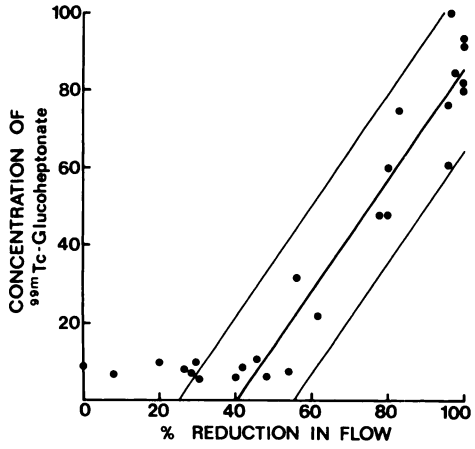


**FIG. 3.** Correlation between bloodflow reduction and uptake of $^{99m}$Tc-glucoheptonate showing positive linear correlation even in tissue with marked reduction in flow (Dog 16) ($r = 0.963$).

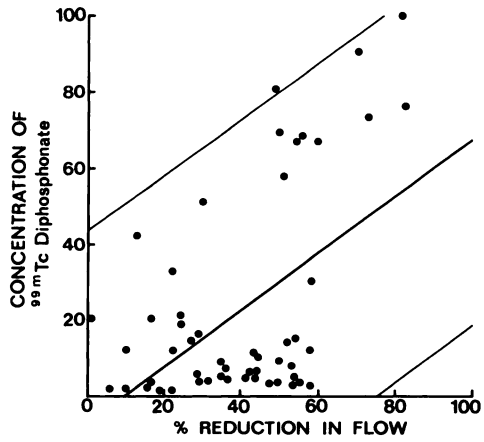


**FIG. 4.** Correlation between bloodflow reduction and uptake of $^{99m}$Tc-diphosphonate ($r = 0.511$). There is marked variability in uptake of $^{99m}$Tc complex at most levels of bloodflow reduction (Dog 3).

bloodflow reduction and uptake of the radiopharmaceutical was seen consistently with all tracers except $^{99m}$Tc-diphosphonate (Fig. 2, Table 2). This linear relationship persisted even at less than 1% of normal blod flow (Fig. 3). With bloodflow reductions smaller than threshold levels, tracer concentration was similar to that in myocardium with normal flow. Correlation was poor in two of six dogs injected with $^{99m}$Tc-diphosphonate (Fig. 4). In these dogs there were tissue samples with high infarct-to-normal concentration ratios but with normal blood flow, and conversely, normal uptake in areas where flow was reduced to 40% of normal.

The degree of ischemia necessary to elicit increased tracer concentration was smallest with $^{99m}$Tc-diphosphonate (11 ± 2% reduction in flow) (Table 2). With $^{99m}$Tc-glucoheptonate and $^{99m}$Tc-tetracycline, flow reductions of 39 ± 7% and 40 ± 4%, respectively, were required before increased tracer concentration could be observed.

Histologic study showed that $^{99m}$Tc-tetracycline was selectively taken up by infarcted tissue while $^{99m}$Tc-diphosphonate concentrated in both infarcted and ischemic tissue to a greater extent than in normal tissue (Fig. 5). For ischemic-to-normal myocardium, the concentration ratios for the tetracycline and diphosphonate tracers were 1.2:1 and 5:1, respectively; for infarcted heart the corresponding ratios were 6.7:1 and 24.5:1. The range of concentration ratios between infarcted-to-normal myocardium and ischemic-to-normal myocardium were great with $^{99m}$Tc-diphosphonate (25–52:1 and 1–11:1, respectively), while the range of concentration ratios for infarcted-to-normal myocardium (6–8:1) was less for $^{99m}$Tc-tetracycline.

## DISCUSSION

If criteria critical to scintigraphic imaging are considered alone, the optimal tracer for acute myocardial infarct scintigraphy is the bone agent $^{99m}$Tc-diphosphonate. The concentration within the infarct relative to most surrounding organs and to normal myocardium is substantially higher than for the other radiotracers. The major limitation is the high concentration in bone, which would ordinarily be particularly troublesome in clinical scintigraphy since overlying ribs and sternum could obscure the cardiac shadow. This problem has been overcome with $^{99m}$Tc-pyrophosphate by subtracting the bone activity using computer techniques (*2*).

The technetium complexes with glucoheptonate, and with the various tetracycline analogs, all turned out to be inferior to the diphosphonate and to about the same degree. Gallium-citrate was also poor, with only a 2:1 T/NT ratio. The diphosphonate, moreover, is a more sensitive index of ischemic threat,

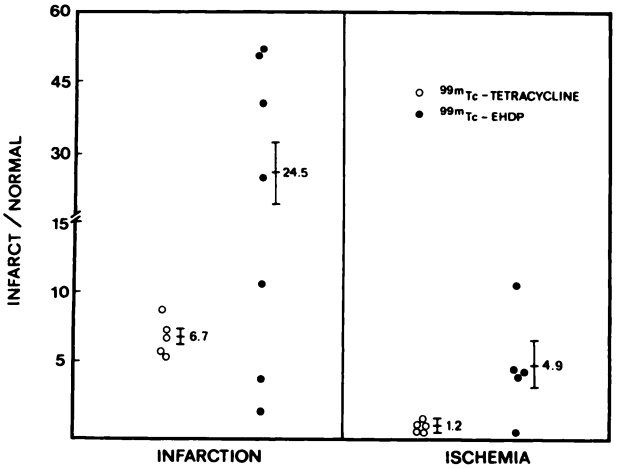


**FIG. 5.** Correlation between ischemia and infarction and uptake of $^{99m}$Tc-tetracycline and $^{99m}$Tc-diphosphonate.

for it signals reversible damage whereas the gluconate and the tetracyclines react only to substantial reductions in blood flow, severe enough to indicate possibly permanent damage.

For all $^{99m}$Tc complexes tested, there was a threshold level in bloodflow reduction below which concentration of the tracer was the same as in histologically normal regions distant from the infarct. At a critical point in bloodflow reduction, the concentration of the radiotracer began increasing in a linear fashion as blood flow was reduced further. This threshold point varied with the radiopharmaceutical. The very small reduction in flow with $^{99m}$Tc-diphosphonate would suggest that this tracer was very sensitive to changes resulting from bloodflow reduction, sufficiently sensitive so that ischemic, reversibly damaged tissue might result in increased tracer concentration. In fact, it is possible that for $^{99m}$Tc-diphosphonate no threshold exists at all since the variability in blood flow throughout the normal left ventricle is approximately 15% (*10*). On the other hand, the myocardial concentration of $^{99m}$Tc-tetracycline and $^{99m}$Tc-glucoheptonate increased only with substantial reductions in flow, suggesting that these tracers concentrate only after significant cellular damage has occurred.

Therefore, if there exists a clinical need to determine the size of a frankly infarcted area, $^{99m}$Tc-tetracycline or glucoheptonate would be the tracers of choice, at least in the current animal model. In an acute coronary episode the diphosphonate, reacting also to minor degrees of ischemia, would include some regions that might recover.

ACKNOWLEDGMENT

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**ERRATUM**

Please note the following correction to the article "Use of Tissue-to-Air Ratio in Computation of Specific Absorbed Fraction" by U. B. Tripathi and P. S. Iyer (*J Nucl Med* 16: 492–494, 1975).

Under the heading "Relationship between TAR and Φ" the paragraph beginning "TAR is extensively used for dose . . ." should read as follows:

"TAR is extensively used for the dose computations in beam therapy (*4*). For point isotropic sources, the tissue-to-air ratio can be defined as

$$\text{TAR} = \frac{\text{Dose to a small mass of tissue in phantom from a point isotropic source}}{\text{Dose to the same mass of the tissue in free space from the same source}} \quad (1)"$$

Consequently, Equations 1 to 4 appearing on page 492 should be redesignated as 2 to 5, respectively.