Tc-99m Dextran: A New Blood-Pool-Labeling Agent for Radionuclide Angiocardiography

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We have explored the possibility of imaging the cardiac blood pool with dextran (Dx) labeled with Tc-99m (Tc) after Sn^{2+} reduction. Stannous dextran (SnDx) kits were prepared in advance and labeling was performed by adding Tc-99m. The labeling efficiency was greater than 95%. Technetium-99m dextran (TcDx) was highly stable both in vivo and in vitro. In seven dogs we compared the quality of blood-pool images obtained with TcDx of different molecular weights (4×10^4 = Dx-40; 5×10^5 = Dx-500; 2×10^6 = Dx-2000) and with Tc-99m red blood cells (TcRBC) labeled in vitro, and determined the organ distribution of this new agent by whole-body scanning and blood sampling. TcDx provided high-quality cardiac blood-pool images up to 60 min after injection. The heart-to-lung ratios averaged 3.7 for TcDx-40, 3.9 for TcDx-500, and 5.4 for TcRBC at 60 min. Whereas TcDx-40 showed a relatively rapid initial urinary excretion and TcDx-2000 was degraded rapidly, TcDx-500 demonstrated the best kinetics for blood-pool imaging. Thus, TcDx is a new radiopharmaceutical with high labeling efficiency and stability. It overcomes a number of the limitations of currently used blood-labeling agents and may become useful for blood-pool imaging in man.

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Noninvasive evaluation of cardiac function with gated equilibrium blood-pool imaging has become an increasingly important tool in clinical cardiology. This technique not only provides noninvasive visualization of cardiac motion and anatomy; it also provides indices of global and regional ventricular performance that are useful in the diagnosis and treatment of cardiovascular disorders (*1–3*). The accuracy of this technique depends to a large extent on the availability of an agent for blood-pool labeling that yields high-contrast images of the cardiac chambers. Ideally such an agent (a) should be stable for hours, the radiolabel remaining tightly bound; (b) should remain in the vascular space; (c) should have a labeling procedure that is simple, fast, and inexpensive; and (d) should provide minimal risk to the patient. Because currently used blood-pool-imaging agents such as Tc-99m-labeled human serum albumin (TcHSA) or red blood cells labeled in vivo and in vitro (TcRBC) do not meet these requirements completely (*4–6*), we labeled dextran (Dx) with Tc-99m and examined its potential for blood-pool imaging. Dx has a long intravascular half-time and is used clinically as a plasma expander (*7,10*). It is the purpose of this communication to describe the labeling procedure and our initial experience in animal experiments with this new radiopharmaceutical for equilibrium blood-pool imaging.

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MATERIALS AND METHODS

Radiopharmaceutical preparation. Technetium-99m was attached to Dx after reduction with stannous ion (Sn) in a deoxygenated aqueous solution. To facilitate

routine use, SnDx reagent kits were formulated so that the radiotracer could be obtained simply by adding pertechnetate. The SnDx kits were prepared in ten-unit batches by dissolving 1.5 mg of $SnCl_2$ in 50 μl of concentrated HCl. During vigorous mixing, 10 ml of a deoxygenated aqueous solution containing 1.0 g of Dx were then added. The Dx used in this study was of various molecular weights,* i.e., 4×10^4 (Dx-40); 5×10^5 (Dx-500), and 2×10^6 (Dx-2000). The Dx solution was deoxygenated by bubbling nitrogen gas through it at 50 ml/min for at least 1 hr. Under aseptic conditions, 1.0-ml aliquots of the SnDx reagent solution was then dispensed through a sterile 0.22-μm membrane filter into 5-ml vials, which were stoppered and sealed in a nitrogen atmosphere and stored at 2-4 °C until used. Before a study, freshly eluted Tc-99m was added to the vial, and the solution was carefully mixed and left at room temperature for 5 min. The labeling efficiency was measured by thin-layer chromatography (TLC) using cellulose polyacetate† and 0.9% NaCl, and/or by paper chromatography (PC) using methyl ethyl ketone (MEK). Blood-pool images obtained with labeled Dx (TcDx) of various molecular weights were compared with those obtained with TcRBC labeled in vitro as described by Smith and Richards (*6*).

Imaging. The suitability of TcDx for blood-pool imaging, its body distribution, and its changes over time were examined in seven mongrel dogs weighing 25-31 kg and anesthetized with pentobarbital (25 mg/kg). In each dog, 0.3 mCi/kg of TcDx-40, TcDx-500, TcDx-2000, or TcRBC were administered intravenously. The body distribution of TcDx-40 was evaluated with a whole-body scanner‡ in one dog in the supine position 20 min after injection. In three animals, gated cardiac blood-pool images obtained with TcDx-40 and TcDx-500 were compared with those obtained with TcRBC. Gated blood-pool images of the heart were recorded at 15-min intervals for 120 min in the left lateral projection, with a scintillation camera equipped with a high-resolution collimator and interfaced to a dedicated minicomputer. The cardiac cycle was divided into 16 frames and imaging continued until each frame contained at least 300,000 counts. The field of view in these studies always included the liver. Time-activity curves were generated from regions of interest on the end-diastolic frame assigned to the left ventricle, on the liver, and on adjacent lung tissue.

The effect of molecular size on the in vivo kinetics of TcDx was evaluated in three dogs. In these experiments, the same camera-computer system was used but was equipped with a diverging collimator. Target-to-collimator distance was 80-100 cm. The field of view encompassed the chest, abdomen, and proximal extremities, and included more than 90% of the total administered activity. Images containing 500,000 counts were obtained in 15-min intervals for 150 min. Time-activity curves were created from regions of interest assigned to the central blood pool, the liver, and the urinary bladder. For normalization between animals, the counts recorded from each region were expressed as a percentage of the total recorded counts. In addition, serial blood and urine samples were obtained for well counting and chromatographic analysis. At the end of each study, the dog's neck was imaged to check for thyroid uptake of free Tc-99m.

RESULTS

Radiochemical purity. The freshly prepared TcDx remained at the origin when it was analyzed by PC using MEK, whereas a peak at or near the solvent front was observed using TLC with 0.9% NaCl. These results are illustrated in Fig. 1 for TcDx-40 and TcDx-2000. Staining of the TLC strips with toluidine blue in methanol verified that the radioactivity was found in the same location as Dx in each preparation. Overall labeling efficiencies were greater than 95%. Analyses of urine samples collected at 4 hr after injection of TcDx-40, TcDx-500, and TcDx-2000, using PC with methanol and 0.9% NaCl, demonstrated little free Tc-99m but also failed to show the presence of TcDx of high molecular weight. The results shown in Fig. 2 are consistent with the presence of low-molecular-weight TcDx.

In vivo kinetics. Figure 3 shows a whole-body scan of a dog 20 min after injection. Most of the activity is present in the central circulation, the liver, and the uri-

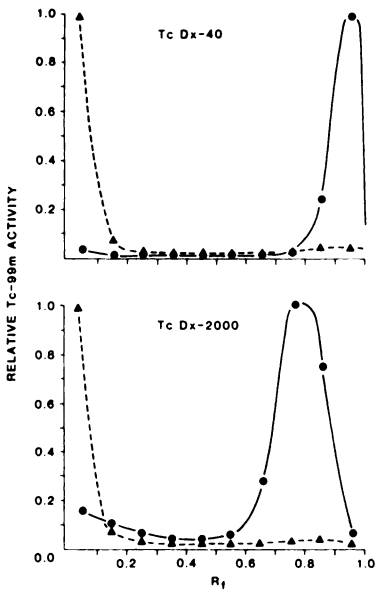

FIG. 1. Paper chromatograms using methyl ethyl ketone (dashed line), and thin-layer chromatograms using 0.9% NaCl on cellulose polyacetate (solid line), of Tc-99m-labeled dextran with molecular weights of 4×10^4 (TcDx-40) or of 2×10^6 (TcDx-2000). These show only very small amounts of pertechnetate, and the labeling efficiency is greater than 95%. There is no evidence of Tc-99m colloid formation.

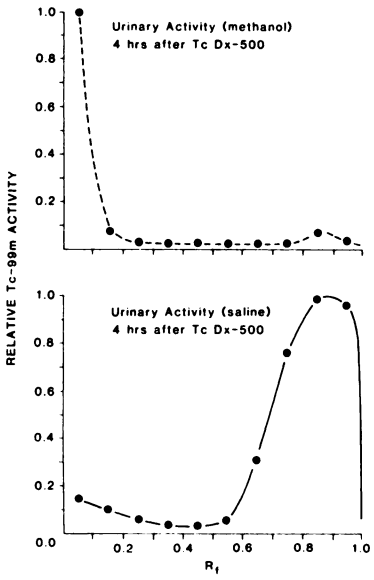

FIG. 2. Paper chromatograms, using methanol and 0.9% NaCl, of urinary radioactivity collected 4 hr after i.v. injection of TcDx-500. They show very little pertechnetate. In saline, broad peak at R_f = 0.8–1.0 is consistent with renal excretion of a low-molecular-weight TcDx to which Tc-99m was still bound.

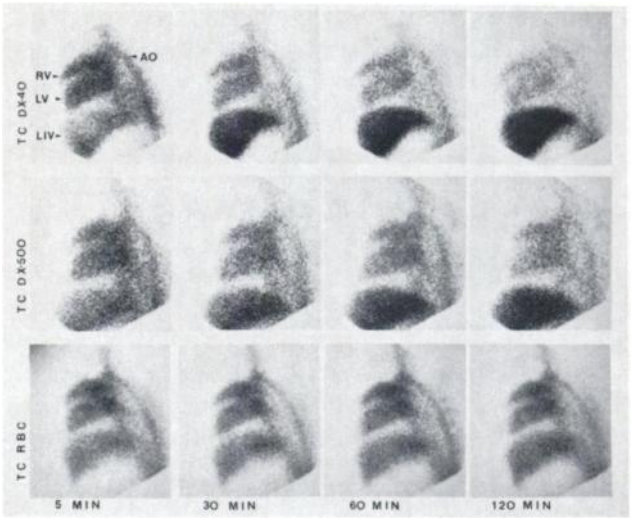

FIG. 4. End-diastolic images obtained in three dogs 5–120 min after injection of TcDx-40, TcDx-500, and RBC labeled in vitro with Tc-99m. Compared with TcRBC images, there is equally good visualization of left (LV) and right ventricles (RV) and aorta (AO) with TcDx during first 60 min after injection. At 120 min, however, TcDx blood-pool images have lost quality. (Abbreviations as in Fig. 1, LIV = liver.)

nary bladder. The heart and major vessels are visualized well, and no activity is noted in the thyroid gland.

TcDx-40 and TcDx-500 provided adequate images for cardiac studies up to 60 min following i.v. injection. As seen in Fig. 4, the resulting blood-pool images are comparable in quality with TcRBC blood-pool images. Five minutes after injection, the heart-to-lung ratios averaged 5.4 for TcDx-40, 5.1 for TcDx-500, and 6.4 for TcRBC, as shown in Fig. 5. These ratios, however, decreased with time as blood activity fell and liver activity increased, so that visualization of the cardiac chambers was inferior to that with TcRBC. During the first 60 min, the target-to-background ratios fell by 32% for TcDx-40, by 23% for TcDx-500, and by 16% for TcRBC. A similar trend was noted for the heart-to-liver ratios, which decreased by 60% for TcDx-40, by 35% for TcDx-500, and by 14% for TcRBC.

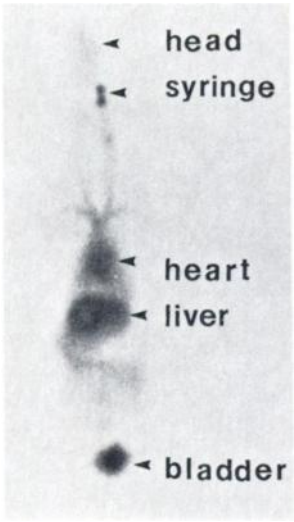

FIG. 3. Whole-body scan of dog 10–20 min after i.v. injection of TcDx-40. Most of the activity is present in central circulation, liver, and urinary bladder. There is good visualization of heart and large vessels. No activity is noted in thyroid or bone marrow.

The effect of molecular weight on the kinetics of TcDx is shown in Fig. 6. TcDx-40, with rather low molecular weight, was rapidly excreted by the kidneys, as demonstrated by the rapid accumulation of activity in the urinary bladder (4–24% of total activity within the first 60 min), whereas liver activity increased only 19–23% within the same interval. By contrast, TcDx-500 accumulated slightly more rapidly in the liver (19–27% within the first 60 min) but was excreted less into the urine, since activity in the bladder increased only by 2% in 60 min. The TcDx-2000, with ultra-high molecular weight, demonstrated an initial liver uptake of 16–20% in the first 60 min, whereas urinary excretion was similar to that of TcDx-40. The changes in blood activity in these three dogs, as measured by well counting of blood samples, gave time-activity curves nearly identical to those obtained from regions of interest over the heart. These observations suggest that labeled Dx with a molecular weight of 500,000 was best for blood-pool imaging in these animal experiments.

Centrifugation of blood, and counting of plasma and whole-blood samples, showed that 88.7 ± 4.5% (n = 14) of the activity in whole blood was present in the plasma fraction. This was similar for all three types of Dx tested, and this fraction remained constant over a 2-hr period. These findings indicate that little if any Tc-99m becomes attached to red blood cells, because even after centrifu-

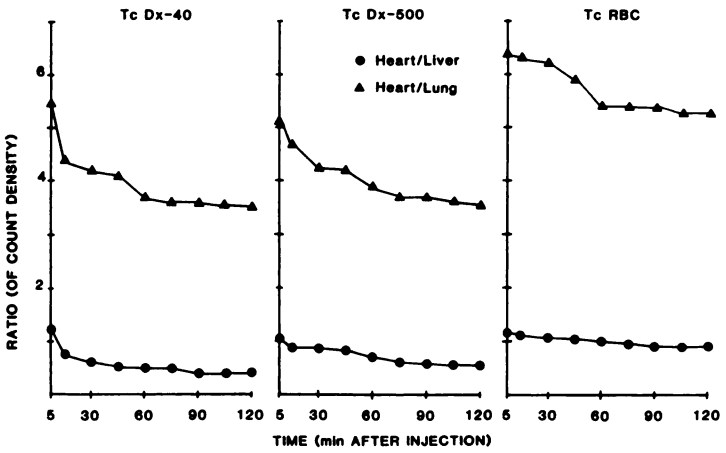

FIG. 5. Comparison of heart-to-liver and heart-to-lung ratios in three dogs after injection of TcDx-40, TcDx-500, and RBC labeled in vitro with Tc-99m. These ratios rapidly declined for TcDx-40, whereas their time course for TcDx-500 was similar to that for TcRBC. (Abbreviations as in Fig. 1.)

gation packed red cells still contain 2-4% plasma (*11*).

Finally, there was no thyroid uptake after TcDx administration, while faint activity in the thyroid gland was present 2 hr after administration of TcRBC. This is further evidence for the high stability of the TcDx complex in vivo.

DISCUSSION

None of the currently used blood-pool-labeling techniques for gated equilibrium cardiac studies is entirely satisfactory. TcHSA leaks out of the vascular space, the labeling efficiency is somewhat variable, and accumulation of the rather large albumin molecules in the liver is relatively high (*5,6,8,9*). This has prompted most laboratories to use RBC labeled in vivo. This technique, however, requires separate injections of (Sn) pyrophosphate and Tc-99m 30 min apart and does not always provide adequate labeling of the blood pool. In our laboratory, 12 out of 88 (13.6%) studies using the in vivo labeling technique were of poor quality, with target-to-background ratios of less than 2.0, so that ejection fraction and regional abnormalities of wall motion could not be accurately assessed. Of these 12 patients, two had sickle cell anemia, three leukemia, two had recent blood transfusions, and one was on heparin. The reason for insufficient labeling remained undetermined in the remaining four patients.

By contrast, in vitro RBC labeling (*6*) results in nearly 100% satisfactory blood-pool labeling in our experience. Target-to-background ratios in 50 consecutive patients were significantly higher with in vitro than with in vivo labeled TcRBC (2.95 ± 0.63 versus 2.44 ± 0.5; $p < 0.01$). Despite these advantages, the labeling procedure is tedious and time-consuming. Approximately 20 to 30 min are required while a single blood sample passes through five different syringes and reaction vials. Hence, there is some risk of contamination and of an accidental change of vials or blood samples when several patients are studied together.

In contrast to in vitro labeled TcRBC with individual preparation of each blood sample, only one batch of TcDx needs to be prepared for several patients. Formulation of the kit for storage and rapid preparation of the blood-pool label are easy and inexpensive. As much as 50 mCi Tc-99m can be attached to 1 ml of 10% SnDx solution for high-count-rate, first-transit radionuclide angiography. The patient is injected only once, and the blood-pool-imaging agent is immediately available.

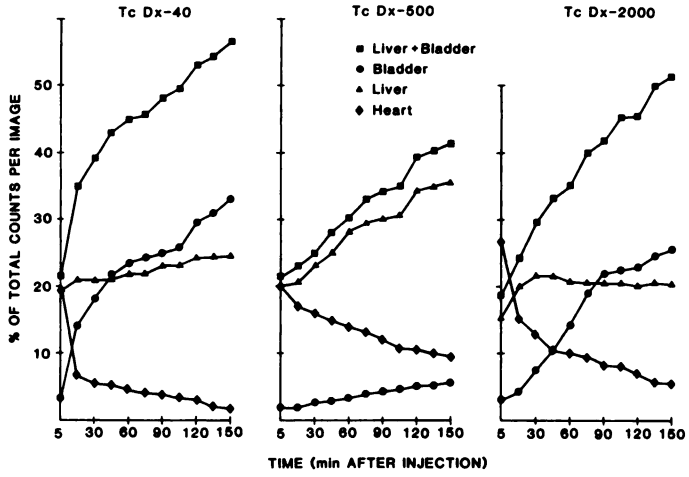

FIG. 6. Relative organ distributions of TcDx-40, TcDx-500, and TcDx-2000 in three dogs. Rapidly falling blood concentrations of TcDx-40 were associated with similar rapid accumulation of activity in urine. TcDx-2000 (ultra-high molecular weight) was more rapidly metabolized by liver and fragments excreted through the kidney, whereas medium-sized molecules showed slowest excretion rates consistent with longest half-time of activity in blood. (Abbreviations as in Fig. 1.)

Chemically and biologically, the labeled Dx demonstrates nearly ideal labeling efficiency and stability. Minimal free Tc-99m was found in the TcDx preparations. In PC analyses with MEK, pertechnetate moves near the solvent front. Little activity is present at the solvent front with PC analysis of freshly prepared TcDx. There is no evidence of significant Tc-99m colloid formation because labeled colloids show no movement from the origin using the TLC system, while TcDx migrates near the solvent front under these analytical conditions (Fig. 1). In this respect, the chemical in vitro characteristics of TcDx are comparable to those of other mono- and polysaccharides, such as glucoheptonate (*12*) or heparin (*13,14*) labeled with Tc-99m using stannous-ion reduction. The binding mechanism of reduced Tc-99m to saccharides is thought to involve formation of a chelate complex, and was recently characterized in detail by De Kieviet (*12*) for glucoheptonate. Accordingly, a similar mechanism can be assumed for TcDx. Two dextrose subunits of the Dx chains form bidentate chelates with reduced Tc-99m, showing the following net stoichiometry:

$$TcO_4^- + 2[C_6H_{10}O_5] + Sn^{2+} + H_2O \rightarrow [TcO(C_6H_8O_5)_2]^- + 4H^+ + [SnO_2(OH)_2]^{2-}.$$

Two adjacent hydroxyl groups per dextrose unit are assumed to be involved in forming the two five-membered chelate rings.

There is no evidence of either free Tc-99m or Tc-99m colloid after intravenous injection, as shown by the absence of thyroid uptake, the lack of bone-marrow activity, and by chromatography of urine samples. However, analysis of urine by PC using methanol and 0.9% NaCl demonstrated the presence of low-molecular-weight TcDx to which the Tc-99m was still bound, as indicated in Fig. 2 by the broad peak in saline medium at R_f = 0.8–1.0. When PC analyses using 0.9% NaCl were performed on the original preparations, only modest migration of activity from the origin was seen. The appearance of low-molecular-weight Dx is consistent with the known metabolic breakdown of Dx (*7,10*). Dx with a molecular weight of less than 40,000 is excreted in man through the kidneys, whereas larger molecules remain in the circulation for weeks and are slowly oxidized. In man, 50% of Dx-40 is excreted through the kidneys within 3 hr (*10*) which, in our experiments, is consistent with the presence of 33% of the TcDx-40 in the bladder 150 min after injection. As a result, the quality of a cardiac image deteriorates over time, which precludes the use of low-molecular-weight Dx for this purpose. On the other hand, Dx-2000 (ultra-high molecular weight) appears to break down more rapidly, as is shown by continuous high liver activity and rapid accumulation of low-molecular-weight Dx in the urine. In our animal experiments, TcDx-500 possessed the best properties for imaging. Both the urinary excretion rate (approximately 2% per hr) and the blood clearance are low. The urinary excretion rate is similar to that in man, which averaged 40% per 24 hr for molecular weights ranging from 70,000 to 150,000 (*10*). Thus, similar pharmacokinetics regarding blood clearance and excretion of TcDx as observed in dogs can be expected in man. Whether purification of Dx to be used in man, from contamination with Dx of low molecular weight, will result in lower initial urinary activity awaits further evaluation.

Use of TcDx in man needs to take into consideration possible side effects of Dx. The known adverse effects on coagulation, kidney function, and the cardiovascular system (*10*), however, seem negligible because of the minimal amount of Dx injected, although occurrence of the known mild allergic reaction following Dx infusion cannot be excluded.

If we assume the biologic behavior of TcDx in man to be similar to that found in dogs, the resulting blood-pool images are of slightly lower quality when compared with images after RBC labeled in vitro. This difference, however, is especially apparent 60–120 min after injection. It is conceivable that once Dx essentially free of contamination with lower-molecular Dx is used, the half-time of TcDx in blood will increase and enhance the image quality. As opposed to TcRBC, this new agent for blood-pool imaging may offer several advantages and hence become a viable alternative for blood-pool imaging. As contrasted with the 20–30 min required for in vitro tagging of RBC, labeling of the pretreated Dx kits requires only 1–2 min. This can be important when time is of the essence or in laboratories with high patient volume, where TcDx has an additional advantage in that several patient doses can be prepared from one batch simultaneously, whereas for TcRBC each injection dose has to be prepared individually. Finally, TcDx may even be superior in instances where TcRBC yield low-quality images, such as in patients with hematologic disorders.

FOOTNOTES

* Pharmacia-Laboratories, Piscataway, NJ.
† Sephraphore III, Gelman Instrument Co., Ann Arbor, MI.
‡ Searle Pho/Con Multi-Plane Imager.

ACKNOWLEDGMENT

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