

KIT-PRODUCED ^{99m}Tc -LABELED RED CELLS FOR SPLEEN IMAGING

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A new method for labeling autologous red blood cells (RBC) with ^{99m}Tc -pertechnetate is presented. RBC are pretreated with stannous glucoheptonate, then mixed with $^{99m}\text{TcO}_4^-$, and finally heat-damaged (H-D). The rapid splenic uptake and high spleen/liver ratio of ^{99m}Tc -H-D RBC yields good-quality spleen images by 30 min postdose.

Radionuclide spleen imaging is widely performed in the course of liver imaging employing ^{99m}Tc -labeled sulfur colloid (1-4). Although this procedure is adequate in most instances (5-7), the spleen image may be unclear when the liver is enlarged or if the area possibly containing splenic tissue is overlapped by the liver image (7). Thus, an agent specific for spleen imaging would be desirable. Such an agent would avoid the undesirable radiation exposure to the liver which occurs when sulfur colloid is employed for specific spleen imaging.

The traditional method for specific spleen imaging employs ^{51}Cr -tagged autologous heat-damaged red blood cells (H-D RBC) (8-10). This method is photon flux and radiation-dose limited and the ^{51}Cr principal photon (320 keV) is not ideal for Anger camera imaging.

We have developed a kit-oriented method for ^{99m}Tc -RBC labeling based on the pretinning approach (11-12). The method replaces stannous citrate (12) which must be freshly prepared with the commercially available stannous glucoheptonate. The result is a procedure for labeling RBC with ^{99m}Tc which is simpler than previously reported methods (13,14) and can be accomplished in any laboratory capable of ^{51}Cr -RBC labeling.

MATERIALS AND METHODS

The basic materials required include: (A) sodium glucoheptonate 200 mg and 0.1 mg stannous chloride (stannous glucoheptonate, New England Nu-

clear No. NRP-180), (B) blood-labeling container/bag (Unitag, Abbott No. 7780), (C) EDTA 5% solution (20 ml of Endrate solution, Abbott ampule No. 6940 aseptically diluted with 40 ml of preservative-free 0.9% NaCl for injection), (D) bucket adapter for centrifuge to contain Unitag bag, and (E) constant-temperature water bath for preparation of H-D RBC. The following methods were used:

I. RBC labeling. (Aseptic technique and radiation shielding must be maintained.)

1. Draw 10 ml of patient's blood in a 20-ml sterile heparinized syringe.
2. Transfer whole blood to a Unitag bag containing 2 ml of ACD solution.
3. Dissolve the contents of a stannous glucoheptonate vial in 1 ml of preservative-free 0.9% NaCl solution for injection. Add the entire dissolved contents of the vial to the Unitag bag containing the blood. Mix carefully and allow to incubate at room temperature for 5 min.
4. Add 1 ml of 5% EDTA solution to the Unitag bag and mix gently.
5. Add 10 ml of 0.9% NaCl for injection to the Unitag bag. Mix gently and centrifuge at 2,000 rpm for 5 min.
6. Remove supernate and discard. Add 20 ml of 0.9% NaCl for injection to the Unitag bag and mix gently. Centrifuge at 2,000 rpm for 5 min. Remove supernate and discard.
7. Employing adequate shielding, add up to 5 ml of sodium pertechnetate ^{99m}Tc in 0.9% NaCl to the RBC in the Unitag bag. Mix gently and incubate 10 min at room temperature for complete labeling.

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II. Heat-damaged red blood cell (H-D RBC) preparation. Transfer labeled RBC to a 20-ml sterile vial and heat in a constant-temperature water bath at 49°C for 15 min with occasional gentle agitation.

III. Determination of labeling efficiency.

1. Transfer an aliquot of ^{99m}Tc -RBC or H-D RBC containing about 200 μCi to a 15-ml vacutainer containing 10 ml of 0.9% NaCl solution.
2. Mix gently and centrifuge at 2,000 rpm for 5 min.
3. Carefully pipet off all supernate and transfer to a clean vacutainer. (Be careful not to remove any RBC).
4. Employing an ion chamber (dose calibrator), determine the activity in the tube containing the RBC and in the tube containing the supernate.
5. Percent labeling is determined by the following: radioactivity in the RBC times 100, divided by the sum of radioactivity in RBC and supernate.

IV. Blood disappearance of ^{99m}Tc -RBC compared with ^{51}Cr -RBC in dogs. Autologous canine RBC were labeled with sodium chromate ^{51}Cr (10). RBC from the same dog were labeled with ^{99m}Tc employing the above Method (I) and then mixed with the ^{51}Cr -RBC in a vial. A sample of this mixture was appropriately diluted to serve as a standard. A known volume of the radiolabeled RBC mixture was injected intravenously into four dogs and blood samples were taken at 10, 20, 30, 60, and 120 min postinjection. The ^{51}Cr and ^{99m}Tc in all samples were measured in a dual-channel well scintillation counter. Corrections were made for Compton scatter and decay. The ratio of ^{99m}Tc to ^{51}Cr counts was determined for each sample. The ^{99m}Tc radioactivity was expressed as a percent of the ^{51}Cr radioactivity by dividing the blood sample ratio of $^{99m}\text{Tc}/^{51}\text{Cr}$ by the standard $^{99m}\text{Tc}/^{51}\text{Cr}$ ratio and multiplying by 100. On one occasion (Dog No. 4) the ^{99m}Tc labeling was done omitting Step 4 in Method I (no EDTA solution was added).

V. Spleen uptake of ^{99m}Tc -H-D RBC in rats. Two 400-gm rats served as homologous blood donors (20 ml whole blood). Ten milliliters were labeled with ^{51}Cr and 10 ml with ^{99m}Tc by Method I. After labeling, the two products were tested for labeling efficiency (Method III) and then the two fractions were mixed in a sterile vial. The mixture of radiolabeled RBC was then heat-damaged (Method II). The radiolabeled mixture was then injected intravenously into fourteen 100-gm male rats (each rat received about 400 μCi of ^{99m}Tc and 2 μCi of ^{51}Cr). The animals were then sacrificed at various time

intervals postinjection. Whole-body gamma camera images were obtained and revealed no significant accumulation of radioactivity as determined visually in organs other than the liver and spleen. These latter organs were removed and weighed. The ^{99m}Tc activity in the liver, spleen, and carcass was determined in an ion chamber. The ^{51}Cr activity in the liver and spleen was determined by counting these organs in a well scintillation counter. The ^{99m}Tc activity in liver, spleen, and carcass (including excreta) were expressed as a percent of the injected dose per whole organ. The spleen-liver ratios were calculated from radioactivity per gram of tissue data for both the ^{99m}Tc and ^{51}Cr (see Table 2).

VI. ^{99m}Tc -RBC in human subjects. Three volunteers on whom a ^{51}Cr -RBC volume determination had been ordered were injected with autologous ^{99m}Tc -RBC immediately upon completion of the ^{51}Cr -RBC procedure. The ^{99m}Tc counts (Compton scatter corrected) from these samples were used to plot a ^{99m}Tc -RBC disappearance curve and determine (by extrapolation to zero time) a red-cell volume.

VII. ^{99m}Tc -H-D RBC spleen images in human subjects. Spleen images were recorded from four patients employing 10 mCi of autologous ^{99m}Tc -H-D RBC. The patients were injected intravenously while lying in the supine position on a plastic-top imaging table. A Pho/Gamma camera detector head was positioned directly beneath the table aimed to include the area of the spleen and a part of the liver and lung bases. All information from the time of injection to 1 hr postinjection was stored on videotape or accepted directly into a Hewlett Packard 5407A computer. Several blood samples were taken during this same period. Rate of organ uptake and spleen/liver ratios were later determined by setting equal-sized areas of interest over each organ. In one of these four patients, ^{51}Cr -RBC (not heat-damaged) were injected immediately prior to the ^{99m}Tc -H-D RBC. Blood samples were taken at intervals up to 61 min. The percent of the ^{99m}Tc -RBC remaining in each of the whole-blood samples could thus be calculated using the unchanging ^{51}Cr -RBC activity level.

RESULTS

Labeling efficiency of ^{99m}Tc -RBC. Over ten batches of human RBC, three batches of canine RBC, and three batches of rodent RBC have been labeled with ^{99m}Tc employing the stannous glucoheptonate method presented. All showed labeling efficiency of the ^{99m}Tc to RBC of greater than 97%. On one occasion the binding was redetermined at 6 and 24 hr following labeling. This resulted in a 96% and 93% labeling efficiency, respectively.

When EDTA was omitted from the procedure (Method I, Step 4) the ^{99m}Tc binding in human RBC was 91% and in canine RBC, 90%.

^{99m}Tc-RBC disappearance in dogs. The results of the comparative whole-blood disappearance of autologous ^{99m}Tc-RBC and ⁵¹Cr-RBC in dogs are shown in Table 1.

^{99m}Tc H-D RBC spleen uptake in rats. The spleen/liver ratios obtained with ^{99m}Tc-H-D RBC and those employing ⁵¹Cr-H-D RBC are presented in Table 2. Close correlation is noted. The high spleen/liver ratios as well as the rate of spleen uptake of the ^{99m}Tc-H-D RBC (30–40% of the injected dose at 30–60 min) suggest that good images can be obtained.

^{99m}Tc-RBC in human subjects. Relatively slow whole-blood disappearance of ^{99m}Tc was found. The ratio of ^{99m}Tc activity concentration at 30 min to that at 10 min was 93% or greater in the three subjects studied. Using the extrapolated counts at time 0 and appropriate standards, the ^{99m}Tc-RBC volume was calculated. The percent differences between the ^{99m}Tc-RBC volume and the sequentially measured ⁵¹Cr-RBC volume were 2%, 7%, and –13%, respectively.

^{99m}Tc-H-D RBC in human subjects. Figure 1 shows a computer-derived printout of splenic uptake in four patients injected intravenously with autologous ^{99m}Tc-H-D RBC. Maximum splenic uptake was assumed to have occurred at 1 hr and was arbitrarily assigned a 100% value. Spleen uptake approaches the maximum value at approximately 30 min postinjection. Spleen-to-liver ratios for equal areas of interest at 60 min ranged from 5.1 to 22 (mean 11). This can be compared with a spleen/liver ratio of less than one for ^{99m}Tc-sulfur colloid in normal subjects using the same technique. The percent of the injected dose (compared with ⁵¹Cr-RBC) remaining in whole blood in one of these patients was 82% at 3.5 min, 39% at 20 min, 16% at 42 min, and 10% at 61 min.

The rapid splenic uptake, high spleen/liver ratio along with rapid blood clearance of the ^{99m}Tc-H-D RBC gives rise to good spleen images at 30–60 min post i.v. injection (Fig. 1).

DISCUSSION

The stannous glucoheptonate method for labeling RBC with ^{99m}Tc can easily be adapted to a "kit" approach, which yields a product of high quality. The advantages of this kit are that all components are commercially available in sterile, pyrogen-free form intended for human use; there is no handling of radioactive supernates and a number of shortened variations of the procedure are possible. Current

TABLE 1. ^{99m}Tc/⁵¹Cr RBC STUDY IN DOGS*

Time postinjection (min)	Dog No. 1 (%)	Dog No. 2 (%)	Dog No. 3 (%)	Dog No. 4† (%)
10	103	95	99	87
20	99	96	99	85
30	101	97	97	84
60	103	93	94	81
120	86	89	88	79

* ^{99m}Tc radioactivity as a percent of ⁵¹Cr in whole blood.
† Method I, Step 4 omitted in labeling procedure.

TABLE 2. H-D RBC STUDY IN RATS

Time of sacrifice post-injection (min)*	Percent of injected dose of ^{99m} Tc-H-D RBC in whole organ			Spleen/liver ratio (activity per gram)	
	Liver (%)	Spleen (%)	Carcass plus excreta (%)	⁵¹ Cr-H-D RBC	^{99m} Tc-H-D RBC
5 (1)	34.4	11.2	54.4	3.8	3.8
	38.6	23.1	38.3	7.5	5.7
10 (3)	31.3	25.6	43.1	7.4	6.9
	32.1	18.6	49.3	4.8	5.3
30 (3)	27.3	37.0	35.8	16.9	16.9
	21.2	28.8	50.0	14.6	14.6
60 (4)	34.1	34.1	31.7	12.5	11.5
	24.6	36.3	39.1	16.0	16.6
120 (3)	26.1	40.6	33.3	19.6	19.8
	15.1	40.5	44.3	22.3	23.1
120 (3)	23.8	45.4	30.8	15.9	14.9
	13.5	45.9	39.0	22.1	24.6
120 (3)	15.8	53.2	31.0	21.4	21.1
	22.4	50.4	27.2	15.0	16.0

* Numbers in parentheses represent number sacrificed.

alternatives are the use of an IMS Mini-jet syringe or a 50-ml disposable syringe with removable plunger to replace the Unitag bag, allowing the drawing of blood and the labeling procedure to be accomplished in the same container. Heat denaturation before the addition of the ^{99m}Tc-pertechnetate shows promise.

Attempts to eliminate the EDTA from the procedure resulted in lower labeling efficiencies. The EDTA may serve to increase the efficiency of RBC washing by removing ionic tin only weakly associated with the RBC membrane.

The procedure as outlined (Method I) consistently gives high labeling efficiency (>97%), which can be shown by in vitro testing (Method III); however, it is important to verify these results in animals. We believe our animal studies corroborate the in vitro studies reported and show a slow rate of disappearance of the ^{99m}Tc-stannous glucoheptonate labeled RBC when not heat-damaged. Such an agent may be

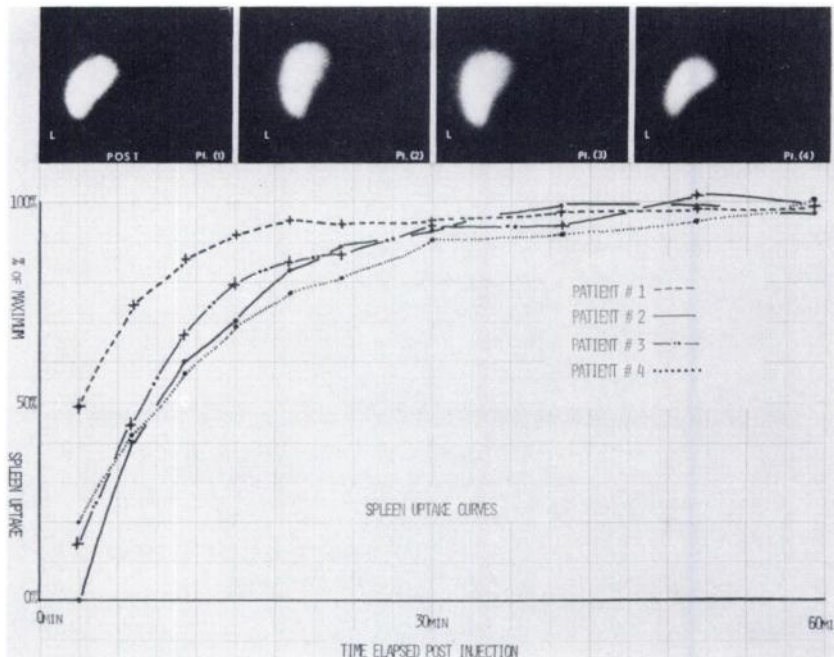


FIG. 1. (Upper) Posterior view, spleen images using ^{99m}Tc -H-D RBC in four patients; (Lower) relative splenic uptake in same patients for first 60 min post i.v. administration of ^{99m}Tc -H-D RBC.

useful in the determination of RBC volume, for blood pool imaging, and for cardiac output studies.

The prepared ^{99m}Tc -H-D RBC showed rapid splenic uptake which compares favorably with the ^{51}Cr -H-D RBC splenic uptake obtained by Wagner and McAfee (8) and the BMHP-damaged ^{51}Cr -RBC splenic uptake reported by Wagner and Weiner (15). The spleen-to-liver ratios in our studies are greater than those reported.

No untoward effects have been observed in either animals or patients following injection of ^{99m}Tc -RBC or ^{99m}Tc -H-D RBC. Stannous glucoheptonate has previously been found to be nontoxic in the clinically used dose range (16). The radiation dose to the normal spleen (critical organ) per millicurie of ^{99m}Tc -H-D RBC administered is about 0.5 rad (assuming 50% uptake). One millicurie suffices for a good imaging study.

ADDENDUM

Since the original submission of this manuscript, it has been found that the RBC washing in Step 6 (see Methods, RBC Labeling) can be eliminated. Using only 1/10 to 1/100 of the stannous glucoheptonate vial contents (2–20 mg of glucoheptonate), it becomes unnecessary to wash the RBC after discarding the first supernate. This approach allows the addition of anywhere from 10 μCi to 100 mCi of pertechnetate ^{99m}Tc to the RBC as explained in Step 7 with preservation of greater than 97% labeling efficiency.

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