EVALUATION OF LABELING PROCEDURES AND IN VIVO STABILITY OF ^{99m}Tc-RED BLOOD CELLS

U. Yun Ryo, Ali A. Mohammadzadeh, Aslam Siddiqui, Lelio G. Colombetti, and Steven M. Pinsky

Michael Reese Hospital and Medical Center and the Pritzker School of Medicine, University of Chicago, Chicago, Illinois

Stable labeling of red blood cells (RBCs) with ^{99m}Tc, in yields of about 66%, was achieved by using stannous chloride as a reducing agent. In a study of 12 patients, the stability of technetium-labeled RBCs in the circulation varied significantly among individuals, but the average biologic half-life of the labeled cells was 16.8 hr. Although the labeling yield was lower when the labeling was carried out without EDTA with the addition of stannous chloride after the pertechnetate, this sequence appears to be the best for labeling RBCs for use in obtaining images of the blood pool or the cardiovascular system because of the increased viability of the RBCs.

The successful labeling of red blood cells (RBCs) with ^{99m}Tc was first reported by Fischer et al (*1*) in 1967. Haubold et al (*2*), using a similar procedure (incubation of the cells with ^{99m}Tc), reported efficient labeling, with yields of 80–90%. The labeling achieved by simple incubation of RBCs with ^{99m}Tc, however, was not stable: Repetitive washing greatly reduced the radioactivity (*3*). The use of stannous chloride during the labeling procedure led to the formation of stable ^{99m}Tc-RBCs in yields of 50–60% (*4,5*). Recent reports indicate that the use of lyophilized stannous citrate or gluconate, in a considerably faster procedure, raises the yield of stable ^{99m}Tc-RBCs to above 90% (*6,7*).

Korubin et al (*5*) and Eckelman et al (*8*) described the advantages of ^{99m}Tc-RBCs over ^{51}Cr-RBCs for blood volume measurements. Blood pool images of high quality were obtained by Haubold et al (*2*) and by Mahon et al (*9*). In recent reports Atkins et al (*10*) and Ryo et al (*11*) showed the usefulness of ^{99m}Tc-RBCs in radionuclide angiography.

The utility of ^{99m}Tc-labeled RBCs in many nuclear medicine procedures has been clearly established by these numerous investigators. However, there still exists wide variation among the labeling procedures and their yields. The stability of these labeled RBCs in vivo, moreover, has not been well studied. The present work was undertaken to evaluate more fully the procedure for labeling RBCs and to determine the stability of the labeled cells in the circulating blood.

METHOD

Labeling. *Procedure A.* Samples of venous blood were drawn into a syringe containing a volume of acid citrate dextrose solution equal to either (A) one-fourth the volume of the blood drawn or (B) the same volume as the blood drawn. Each blood sample was centrifuged for 5 min at 2,000 rpm and the supernatant was withdrawn by use of a sterile syringe and needle.

To the packed RBCs 15–30 mCi of ^{99m}Tc-pertechnetate in 0.5–1.5 ml of saline were added, and the mixture was incubated in a gently shaking bath for 20 min at room temperature (22°C). A 0.1% solution of stannous chloride dihydrate in acid citrate dextrose solution, filtered through a 0.22-micron filter, was added to the mixture of RBCs and pertechnetate in an amount sufficient to produce a concentration of 20–30 μg of stannous chloride dihydrate per milliliter of packed cells. This mixture was again incubated in the bath for 10 min. After this second incubation, the RBCs were washed twice with sterile isotonic saline. The red cells were resuspended in saline or 5% dextrose before being infused into the patient.

Procedure B. Red blood cells were labeled according to the method described by Gutkowski and Dworkin (*7*) by successively adding the contents of one vial of stannous glucoheptonate and 1 ml of 5% EDTA to 10 ml of blood before incubation of the blood with ^{99m}Tc.

Received May 21, 1975; revision accepted Aug. 11, 1975.

For reprints contact: U. Yun Ryo, Michael Reese Hospital and Medical Center, Div. of Nuclear Medicine, 2901 S. Ellis Ave., Chicago, Ill. 60616.

The stannous gluconate (New England Nuclear Corp., Boston, Mass.) contains 0.1 mg of stannous chloride and 200 mg of sodium glucoheptonate in physiologic saline solution.

Animal study. Fifteen mongrel dogs of either sex, weighing 8–15 kg, were used to study the stability of labeled RBCs in vivo.

In the first group of ten dogs, RBCs obtained from six dogs were labeled by Procedure A and those from four dogs by Procedure B. Each preparation of RBCs was infused into the dog from which it had been obtained, and venous blood samples were drawn at various time intervals. The washed RBCs from these samples were counted for ^{99m}Tc activity, which were expressed as percentages of the activity present in the first blood sample, drawn 15 min after infusion of the labeled RBCs.

In a second group of five dogs, 10 ml of venous blood were drawn from each dog and divided in half. One half was labeled with ^{51}Cr using a standard method (*12*), and the other half was labeled with ^{99m}Tc using Procedure A. Each pair of autologous differently labeled samples of RBCs was infused into the dog from which the cells had been obtained, and venous blood samples were drawn after 30 min, 1 hr, 2 hr, and 24 hr. The blood samples were counted immediately for ^{99m}Tc activity (per ml whole blood) and after 4 days for ^{51}Cr activity. The recorded ^{99m}Tc activities were corrected for the scattered ^{51}Cr activity observed at the ^{99m}Tc window.

Study on patients. The study of the stability of ^{99m}Tc-RBCs was extended to 12 patients who had been referred to the Nuclear Medicine Division for blood pool or vascular imaging procedures (*10,11*). None of these patients had any known hematologic disease. Samples (10–15 ml) of venous blood were drawn and the RBCs were labeled by Procedure A. Fifteen minutes after the infusion of ^{99m}Tc-RBCs, a 2–5-ml blood sample was drawn, and from two to four more samples were subsequently drawn at various times during the testing period. An aliquot (1.0 ml) was taken from each of these samples for use in the determination of ^{99m}Tc radioactivities in the RBCs and in the plasma.

In order to compare the labeling yields obtained by the different procedures, Procedure B was used to label RBCs from six of these patients. These labeled RBCs were not reinfused into the patients.

RESULTS

Labeling. After removal of plasma and acid citrate dextrose (ACD) by centrifugation, the amount of plasma contained in the packed RBCs was found to be 27% of the original volume in Group 1 (blood-to-ACD 4/1) and 15% in Group 2 (blood-to-ACD 1/1). The means and standard deviations of the labeling yields by Procedure A were 53.6% (±4.5%) in Group 1 and 66.3% (±2.1%) in Group 2. In the six samples of blood labeled by Procedure B, the mean labeling yield was 90.1% (±0.8%).

In three cases the blood sample was divided between two tubes. During the labeling procedure one tube was incubated at 37°C and the other at room temperature. The temperature differences during incubation had no effect on the labeling yield. In half the cases studied, hemolysis was indicated by a pinkish coloration in the supernatant when the packed cells were washed with isotonic saline after completion of the labeling steps.

In vivo studies. The half-lives of ^{99m}Tc-RBCs labeled by Procedures A and B in the circulating blood of dogs are compared in Table 1. The half-life of ^{99m}Tc-RBCs from Procedure B was much shorter than that from Procedure A.

Table 2 compares the clearance rates of ^{99m}Tc-RBCs (Procedure A) and ^{51}Cr-RBCs from the circulating blood of dogs. During the first 2 hr after infusion, the quantity of ^{99m}Tc-RBCs and ^{51}Cr-

TABLE 1. STABILITY OF ^{99m}Tc-LABELED AUTOLOGOUS RED BLOOD CELLS IN THE CIRCULATING BLOOD OF DOGS

Time after infusion	2 hr	4 hr	16 hr	24 hr
Procedure A*	83.9 (±3.8)	70.5 (±16.1)	53.1 (±3.6)	50.3 (±4.6)
Procedure B*	81.2 (±11.1)	43.2 (±18.4)	16.8 (±5.3)	15.3 (±3.5)

* The figures represent the mean fraction of tagged RBCs recovered from venous blood at time of sampling, expressed as percentage of the activity found in a blood sample drawn 15 min after injection. Standard deviations are given in parentheses.

TABLE 2. COMPARISON OF STABILITY BETWEEN ^{99m}Tc- AND ^{51}Cr-LABELED AUTOLOGOUS RBCs IN THE CIRCULATING BLOOD OF DOGS

Time after infusion	½ hr	1 hr	2 hr	24 hr
^{51}Cr-RBC*	97.5 (±2.6)	91.4 (±3.4)	86.6 (±1.5)	80.2 (±5.4)
^{99m}Tc-RBC*	97.6 (±1.2)	92.1 (±2.8)	83.5 (±3.1)	53.9 (±9.4)

* The figures represent the mean fraction of tagged RBCs recovered from venous blood at time of sampling, expressed as percentage of the activity found in a blood sample drawn at 15 min after injection. Standard deviations are given in parentheses.

RBCs remained the same in the circulating blood, but after 24 hr significantly larger portions of ^{99m}Tc-RBCs had disappeared from circulation ($p < 0.01$).

After an infusion of ^{99m}Tc-RBCs, an average of 96% of the radioactivity in the circulating blood is bound to the RBCs. This percentage was maintained even in a sample drawn 18 hr after the infusion.

The mean clearance rate of ^{99m}Tc-RBCs labeled by Procedure A was calculated from the activities (per ml whole blood) measured in samples of venous blood drawn at various time intervals between 15 min and 10 hr after infusion of labeled cells in 12 patients. The stability of labeled RBCs in the circulation varied markedly among individual patients. The biologic half-life of the ^{99m}Tc-RBCs ranged from 5 to 35 hr with an average of 16.8 (±9.6) hr. The effective half-life of the labeled cells was 4.42 hr.

DISCUSSION

The binding capacity of RBCs for ^{99m}Tc was not quantitatively measured in this study. As a rule, 2 mCi of ^{99m}Tc was added to 1 ml of blood, and the activity of the labeled RBCs could easily have exceeded 1 mCi per ml of blood.

In contrast to the effect of temperature in the labeling of RBCs with chromium (*12*), incubation of the RBC–pertechnetate mixture at 22°C produced the same labeling yield as incubation at 37°C.

Reduced technetium binds readily to the plasma protein (*13*) which will therefore compete with the RBCs for the ^{99m}Tc during incubation. Perhaps the labeling efficiency would be raised further if the plasma was entirely removed at the beginning of the labeling procedure.

The labeling yield was far superior when RBCs were labeled with ^{99m}Tc by Procedure B. When infused into dogs, however, cells labeled by Procedure B disappeared from circulation more rapidly than cells labeled by Procedure A. Procedure B, introduced by Gutkowski and Dworkin for the preparation of an effective spleen-scanning agent (*7*), appears to cause considerable damage to the cells. Procedure A also probably damages the RBCs somewhat, as indicated by the more rapid disappearance—apparent 2 hr after infusion—of these cells than of those labeled with ^{51}Cr (Table 2). Scans of two dogs obtained 24 hr after an infusion of ^{99m}Tc-RBCs labeled by Procedure A showed prominent spleen images and less distinct blood pool images; this finding confirms that sequestration by the spleen plays an important role in the disappearance of ^{99m}Tc-RBCs from the circulation.

Chemical damage of the RBC membrane by mercurihydroxypropane has been reported by Wagner et al (*14*), and the use of a concentrated stannous citrate solution also damaged RBCs, as judged by the rapid uptake of the labeled cells by the spleen (*15*).

In the present study, red blood cells labeled with ^{99m}Tc by Procedure A were found to be fairly stable in the circulation for the first 1–2 hr, which agrees with the findings of Eckelman et al (*8*) and Korubin et al (*5*). The average clearance rate of these labeled cells, measured in 12 patients, was about 3%/hr. However, the cause of the considerable individual variations from this average remains to be discovered. Plausible explanations include individual differences in the fragility of the RBCs, in the degree of damage inflicted on the RBC membranes, and in the rate of elution of technetium from the cells.

The radiation doses absorbed by the whole body and by various organs were calculated from Equation 3d in MIRD Pamphlet No. 1 (*16*) and from the data of Cloutier and Watson (*17*) on the blood mass and relative distribution of blood in various organs. Since the half-life of ^{99m}Tc-RBCs varied significantly among the patients, the radiation dose imposed by an infusion of ^{99m}Tc-RBCs is only predictable within wide limits. Applying the half-life of labeled cells observed in this study, we estimate that the radiation dose from an infusion of 10 mCi of ^{99m}Tc-RBCs to the whole body is 270 mrads on the average, with a range of 176–357 mrads.

Since the radiation dose from 10 mCi of ^{99m}Tc is well within the acceptable range and the quality of blood pool images obtained with 5–10 mCi of the labeled RBCs is excellent (*11*), ^{99m}Tc-RBCs appear to be the best agent currently available for blood pool imaging. Because of probable damage to RBCs by the labeling procedure, the use of ^{99m}Tc-RBCs for a study of RBC life span and blood volume needs to be further evaluated.

REFERENCES

1. FISCHER J, WOLF R, LEON A: Technetium-99m as a label for erythrocytes. *J Nucl Med* 8: 229–232, 1967

2. HAUBOLD U, PABST HW, HÖR G: Scintigraphy of the placenta with ^{99m}Tc-labeled erythrocytes. In *Medical Radioisotope Scintigraphy*, vol 20, Vienna, IAEA, 1969, pp 665–674.

3. WEINSTEIN MB, SMOAK WM: Technical difficulties in ^{99m}Tc-labeling of erythrocytes. *J Nucl Med* 11: 41–42, 1970

4. ECKELMAN W, RICHARDS P, HAUSER W, et al: Technetium-labeled red blood cells. *J Nucl Med* 12: 22–24, 1971

5. KORUBIN V, MAISEY MN, MCINTYRE PA: Evaluation of technetium-labeled red cells for the determination of red cell volume in man. *J Nucl Med* 13: 760–762, 1972

6. SMITH T, RICHARDS P: A simple kit for the rapid preparation of ^{99m}Tc red blood cells. *J Nucl Med* 15: 534, 1974

7. GUTKOWSKI RF, DWORKIN HJ: Kit-produced ^{99m}Tc-labeled red cells for spleen imaging. *J Nucl Med* 15: 1187–1191, 1974

8. ECKELMAN WC, REBA RC, ALBERT SN: A rapid simple improved method for the preparation of Tc^{99m} red blood cells for the determination of red cell volume. *Am J Roentgenol Radium Ther Nucl Med* 118: 861–864, 1973

9. MAHON DF, SUBRAMANIAN G, MCAFEE JG: Experimental comparison of radioactive agents for studies of the placenta. *J Nucl Med* 14: 651–659, 1973

10. ATKINS HL, ECKELMAN WC, KLOPPER JF, et al: Vascular imaging with ^{99m}Tc-red blood cells. *Radiology* 106: 357–360, 1973

11. RYO UY, LEE JI, ZARNOW H, et al: Radionuclide angiography for the detection of aortic aneurysm. *J Nucl Med* 15: 1014–1017, 1974

12. GRAY SJ, STERLING K: The tagging of red cells and plasma proteins with radioactive chromium. *J Clin Invest* 29: 1604–1613, 1950

13. STERN HS, ZOLLE I, MCAFEE JG: Preparation of technetium(Tc^{99m})-labeled serum albumin (human). *Int J Appl Radiat Isot* 16: 283–288, 1965

14. WAGNER HN, WEINER IM, MCAFEE JG, et al: 1-Mercuri-2-hydroxypropane (MHP). A new radiopharmaceutical for visualization of the spleen by radioisotope scanning. *Arch Intern Med* 113: 696–701, 1964

15. ECKELMAN W, RICHARDS P, ATKINS HL, et al: Visualization of the human spleen with ^{99m}Tc-labeled red blood cells. *J Nucl Med* 12: 310–311, 1971

16. LOEVINGER R, BERMAN M: A schema for absorbed-dose calculations for biologically distributed radionuclides. MIRD Pamphlet No 1, *J Nucl Med* 9: Suppl No 1, 7–14, 1968

17. CLOUTIER RJ, WATSON EE: Radiation dose from radioisotopes in the blood. In *Medical Radionuclides, Radiation Dose and Effects*, Oak Ridge, Tenn., USAEC, 1970, pp 325–346