A Comparative Evaluation of Techniques for Rapid and Efficient In Vivo Labeling of Red Cells with [^{99m}Tc] Pertechnetate

Robert G. Hamilton and Philip O. Alderson

Armed Forces Radiobiology Research Institute, Bethesda, Maryland, and Johns Hopkins Hospital, Baltimore, Maryland

Red blood cells (RBCs) labeled in vivo with $^{99m}TcO_4^-$ have recently been recommended for blood-pool imaging, but the optimum conditions for in vivo labeling of RBCs have not been clearly defined. We therefore evaluated several stannous-ion preparations and stannous-ion concentrations to determine which provided the best labeling. The effect of the time interval between the Sn(II) and $^{99m}TcO_4^-$ injections and the effect of carrier technetium on labeling efficiency were also studied. Maximal in vivo labeling efficiency was obtained using an intravenous dose of 10 μg Sn(II)/kg followed 5–30 min later by an injection of $^{99m}TcO_4^-$. Neither the chelated form of stannous ion used in these studies nor the amount of carrier present had a significant effect on labeling efficiency. The biologic half-time of Tc-99m RBCs labeled in vivo was similar to that of Tc-99m RBCs labeled in vitro. In vivo labeling is a rapid and efficient method for the preparation of Tc-99m RBCs.

J Nucl Med 18: 1008–1011, 1977

The phenomenon of in vivo labeling of red blood cells (RBCs) with $^{99m}TcO_4^-$ following the intravenous administration of stannous ion has been reported by several investigators (*1–4*). Recent reports (*5,6*) suggest that RBCs labeled in this manner may be clinically useful for large-vessel imaging, including cardiac blood-pool studies. The optimum conditions for labeling RBCs in vivo, however, have not been clearly defined in terms of the best stannous ion agent, the optimal stannous-ion concentration, the best interval between Sn(II) and $^{99m}TcO_4^-$ injections, and the effect of carrier technetium on the efficiency of labeling RBCs in vivo. The current study was undertaken to answer these questions and to compare Tc-99m RBCs labeled in vivo with those labeled in vitro in terms of labeling efficiency, biologic half-times, and image quality.

METHODS

Fifteen vials each of stannous diphosphonate*,†, stannous pyrophosphate‡, and stannous tartrate§ were obtained. Three vials from each group were examined for actual stannous-ion concentration by a potentiometric iodine titration test (*7*) before and after the period of experimentation. Stannous ion [Sn(II), molecular weight 118.7] was then administered to Sprague-Dawley rats (325 ± 25 g) on a weight basis—micrograms of Sn(II) ion per kilogram. In each experiment, 6–7 ml of whole blood were withdrawn from the experimental animal 2 hr after tracer injection. Two 1-ml aliquots of whole blood were removed from the sample and the remaining 4–5 ml of blood were centrifuged. After centrifugation, plasma was decanted and the packed RBCs were washed in 7 ml of saline. The two 1-ml aliquots of whole blood, 1 ml of plasma, and approximately 1 ml of washed RBCs were pipetted into separate weighed gamma-counting vials, and counted in a

Received Jan. 31, 1977; revision accepted May 25, 1977.

For reprints contact: Lt. Hamilton, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

gamma well counter against appropriate standards to determine the % ID/g in whole blood, plasma, or RBCs. Carefully pipetted 1-ml aliquots of whole blood, plasma, and RBCs were weighed, and appropriate conversion factors determined to allow the data to be expressed as % ID/ml. The mean rat whole-blood volume was then experimentally determined (n = 5) by measurements of I-125 HSA plasma volume and autologous Cr-51 RBC red cell volume. The % ID/ml of packed RBCs was multiplied by the red-cell volume to determine % ID in the total-body red-cell pool.

Preliminary experiments demonstrated that 30 min to 2 hr after in vivo labeling of RBCs, approximately 90% of the injected dose remained in the blood pool. Control animals receiving only $^{99m}TcO_4^-$ retained roughly 11% of the injected dose (10% ID plasma-associated and 1% ID bound to RBCs) in whole blood at 2 hr. These results and the potential clinical interest in the in vivo stability of the RBC label led us to choose 2 hr postinjection as the sampling time in experiments to determine the optimal stannous-ion concentration, the best stannous-ion agent, and the effect of carrier Tc-99. Significance testing of the experimental results was performed using Dunnett's analysis of variance test (*8*). The following experiments were performed:

1. Determination of optimal stannous-ion concentration. Rats (n = 72, twelve per group) received femoral vein injections of varying concentrations (1, 2, 5, 10, 20, or 40 μg/kg) of stannous ion in the form of stannous pyrophosphate, followed 5–15 min later by 3 mCi of $^{99m}TcO_4^-$.

2. Determination of optimal time interval between Sn(II) and $^{99m}TcO_4^-$ injections. Rats (n = 20, four per group) received a femoral vein injection of 10 μg Sn(II)/kg in the form of stannous pyrophosphate. After varying time intervals (1, 5, and 30 min, 2 and 24 hr), each animal received 3 mCi of $^{99m}TcO_4^-$.

3. Comparison of stannous-ion preparations. Rats (n = 40, ten per group) received femoral vein injections of 10 μg Sn(II)/kg as diphosphonate, pyrophosphate, or tartrate, followed 5–10 min later by 3 mCi of $^{99m}TcO_4^-$.

4. Determination of carrier [^{99m}Tc] pertechnetate effect on in vivo labeling of ^{99m}Tc-RBCs. Rats (n = 24, six per group) were injected with 10 μg Sn(II)/kg in the form of stannous pyrophosphate, followed 5–10 min later by 3 mCi of $^{99m}TcO_4^-$ from eluates that contained increasing amounts of Tc-99, originating from the decay of 10, 20, 30, or 50 mCi of molybdenum-99 (*9*).

5. Comparison of Tc-99m RBCs labeled in vivo and in vitro. In vitro labeling was performed using the Smith and Richards Kit method (*9*). The in vivo method employed a 10-μg Sn(II)/kg dose of pyrophosphate injected 5–10 min before $^{99m}TcO_4^-$. Samples of whole blood were drawn from rats (n = 44, four per group) at varying times (15 and 30 min, 1, 2, 6, 12, 18, 24, 28, 36, and 48 hr) after a 3-mCi intravenous injection of $^{99m}TcO_4^-$ or Tc-99m RBCs. The labeling efficiency and biologic half-time of each preparation was determined after appropriate decay corrections.

Precordial images were then obtained in five beagle dogs using Tc-99m RBCs labeled in vivo or in vitro. To minimize the "cross over" effect of tin, each dog first received 5 mCi of in vitro-labeled Tc-99m RBCs prepared with less than 1 μg of Sn(II). Four days later, each dog received the larger dose of Sn(II) (e.g., 120 μg per 12-kg dog) required for in vivo labeling, followed 5–10 min later by a 5 mCi dose of $^{99m}TcO_4^-$. Gamma-camera images of the precordial region (999,000 counts) were taken 10, 20, 30, 60, and 120 min after injection using a low-energy converging collimator.

RESULTS

The red-cell-labeling efficiency (% ID/ml RBCs) obtained with different doses of stannous ion is displayed in Fig. 1. Red-cell activity obtained with 10 μg Sn(II)/kg was significantly greater ($p < 0.001$) than that obtained with 1–5 μg Sn(II)/kg, but further increases in the stannous-ion dose (up to 40 μg Sn(II)/kg) did not result in additional increases in red-cell activity. Maximal RBC labeling was obtained when the time interval between injection of Sn(II) and $^{99m}TcO_4^-$ was 5–30 min (Fig. 2). There was no significant difference in the efficiency of RBC labeling obtained using any of the four Sn(II) ion preparations tested (Table 1), or using $^{99m}TcO_4^-$ containing small amounts of carrier Tc-99. Two

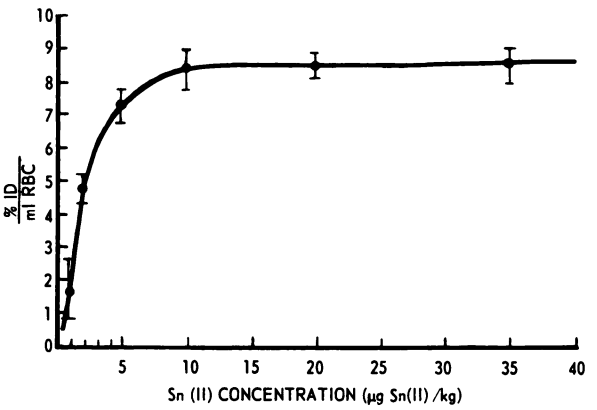

FIG. 1. Tc-99m RBCs labeled in vivo. Percent injected dose per ml of RBCs as function of Sn(II) ion concentration. Results are given as % ID/ml RBC ± 1 S.D.

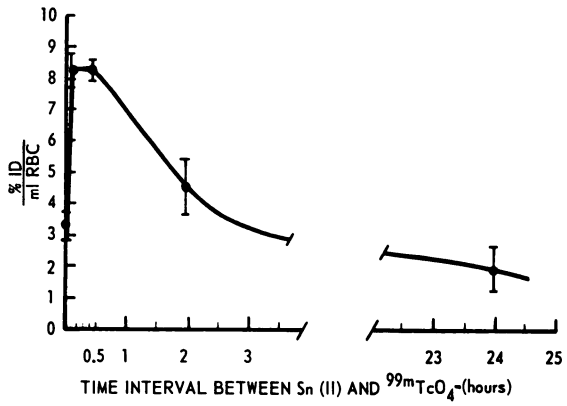

FIG. 2. Tc-99m RBCs labeled in vivo. Percent injected dose per ml of RBCs as a function of time interval between Sn(II) and $^{99m}TcO_4^-$ injections. Results are given as % ID/ml RBC ± 1 S.D.

hours after injection, 85% (±2%, S.D.) of the injected dose remained in the blood pool.

Animals receiving Tc-99m RBCs labeled in vitro demonstrated slightly more RBC activity than animals that underwent labeling in vivo, but the differences were not statistically significant (Table 2). In rats the biologic half-time of red cells labeled in vivo (19.5 hr) was similar to that of red cells labeled in vitro (21.0 hr). Precordial gamma-camera images in dogs showed clear visualization of the cardiac blood pool and other large arteries by both agents (Fig. 3). There was no perceptible degradation in the quality of the images obtained with either agent between 20 and 120 min after injection.

DISCUSSION

Previous reports of blood-pool imaging with in vivo-labeled Tc-99m RBCs (*5,6*) have differed greatly with respect to the amount of Sn(II) ion employed and the time interval between Sn(II) ion and pertechnetate injections. In the present studies, 10 μg Sn(II)/kg was found to be the minimum dose of stannous ion that resulted in satisfactory RBC labeling (85 ± 2% bound to RBCs). This dose is less than the previously reported "threshold value" for labeling [20 μg Sn(II)/kg] (*4*) and much less than the 200 μg/kg dose employed by Pavel et al. (*6*) in patients. The minimum amount of Sn(II) necessary to obtain satisfactory in vivo labeling of RBCs with Tc-99m should be used due to the potential toxicity of intravenously injected tin. McRae et al. (*1*) report that Sn(II) doses of 2 mg Sn(II)/kg or less produced no observable ill effects in rodents. This suggests a safety factor of roughly 200:1 with the use of Sn(II) concentrations of 10 μg/kg to prepare in vivo-labeled Tc-99m RBCs.

In the current study, maximum in vivo labeling of red cells occurred when $^{99m}TcO_4^-$ was injected 5–30 min after Sn(II) ion. In the study of Stokely et al. (*5*), there was a 24-hr interval between the stannous-ion and $^{99m}TcO_4^-$ injections. They used this long interval to allow decay of activity from a "hot spot" infarct image performed with Tc-99m Sn pyrophosphate. The RBC-to-plasma activity ratios achieved in their study (21:1) were lower than those obtained with stannous pyrophosphate in the current study (43:1, Table 1). This is predictable, since there is decreased labeling efficiency in vivo after this interval (Fig. 2). Pavel et al. (*6*) studied the efficiency of in vivo red-cell labeling 30, 60, and 120 min after Sn-PP_i injection in patients. They found maximum labeling efficiency (88%) at 30 min, but shorter time intervals were not studied, as they felt at least 10–15 min were necessary for adequate Sn-PP_i mixing in the blood pool. The current results indicate that adequate labeling can be obtained as early as 5 min after Sn-PP_i injection in rats, but a 10–15 min Sn-PP_i mixing period may be necessary in patients.

CONCLUSION

The results of this study indicate that differences in red-cell labeling by the in vivo and in vitro techniques are statistically insignificant, and that blood-

TABLE 1. EFFICIENCY OF IN VIVO RED CELL LABELING USING FOUR STANNOUS ION PREPARATIONS*

Agent	n	Whole blood	RBCs	Plasma
Diphosphonate†	10	4.31 ± 0.24	8.57 ± 0.59	0.18 ± 0.02
Diphosphonate‡	10	4.24 ± 0.21	8.42 ± 0.41	0.14 ± 0.04
Pyrophosphate	10	4.28 ± 0.24	8.64 ± 0.58	0.20 ± 0.02
Tartrate (lyophilized)	10	4.13 ± 0.25	8.24 ± 0.72	0.16 ± 0.04

* Results given in % ID/ml ± 1 S.D., 2 hr following pertechnetate injection.
† Osteoscan.
‡ HEDP.

TABLE 2. EFFICIENCY OF Tc-99m-RBC LABELING BY IN VITRO AND IN VIVO METHODS*

Method	n	Whole blood	RBCs	Plasma
In vivo	10	4.28 ± 0.24	8.64 ± 0.58	0.20 ± 0.02
In vitro	10	4.50 ± 0.19	9.11 ± 0.49	0.22 ± 0.03

* Results are given in % ID/ml of whole blood, RBCs, and plasma, 2 hr after injection.

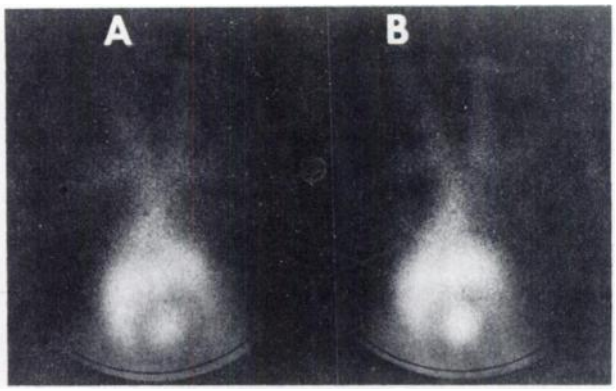

FIG. 3. Precordial images (999,000 counts) performed with Tc-99m RBCs labeled by (A) in vivo (Sn-PP$_i$) and (B) in vitro (BNL kit) methods. Images were obtained 30 min after injection of activity. In each image left ventricle is clearly visible.

pool images of good quality can be obtained with either method. These results were obtained 2 hr post-injection, indicating that in vivo Tc-99m RBCs can provide blood-pool images of high quality at least that long after injection. The findings suggest that RBCs labeled in vivo with Tc-99m may be as useful as those labeled in vitro for serial ejection-fraction studies (*10*) and large-vessel imaging (*11*). In vivo labeling of Tc-99m RBCs is quicker and more easily performed than the in vitro procedures. If further trials confirm the initial clinical results (*5,6*), Tc-99m RBCs labeled in vivo may become a widely used agent for blood-pool imaging.

ACKNOWLEDGMENTS

We wish to thank Dr. William C. Eckelman for providing facilities for the potentiometric iodine titration analysis of our Sn(II) samples, and Nelson Fleming and Thomas Peters for their excellent technical support.

FOOTNOTES

* HEDP, Union Carbide, Tuxedo, N.Y.
† Osteoscan, Procter & Gamble, Cincinnati, Ohio.
‡ Technescan PYP™, Mallinckrodt, St. Louis, Mo.
§ Union Carbide, Tuxedo, N.Y.

REFERENCES

1. McRae J, Sugar RM, Shipley B, et al: Alterations in tissue distribution of ^{99m}Tc-pertechnetate in rats given stannous tin. *J Nucl Med* 15: 151–155, 1974

2. Chandler WM, Shuck LD: Abnormal technetium-99m pertechnetate imaging following stannous pyrophosphate bone imaging. *J Nucl Med* 16: 518–519, 1975 (Abst)

3. Walker AG: Effect of Tc-99m Sn bone scan agents on subsequent pertechnetate brain scans. *J Nucl Med* 16: 579, 1975 (Abst)

4. Khentigan A, Garrett M, Lum D, et al: Effects of prior administration of Sn(II) complexes on *in vivo* distribution of ^{99m}Tc-pertechnetate. *J Nucl Med* 17: 380–384, 1976

5. Stokely EM, Parkey RW, Bonte FJ, et al: Gated blood pool imaging following ^{99m}Tc stannous pyrophosphate imaging. *Radiology* 120: 433–434, 1976

6. Pavel DG, Zimmer AM, Patterson VN: *In vivo* labeling of red blood cells with ^{99m}Tc: A new approach to blood pool visualization. *J Nucl Med* 18: 305–308, 1977

7. Meites L, Thomas HC: Potentiometric measurements. In *Advanced Analytical Chemistry*, New York, McGraw-Hill, 1958, pp 56–64

8. Dunnett CW: A multiple comparison procedure for comparing several treatments with a control. *J Am Stat Assoc* 50: 1096–1121, 1955

9. Smith TD, Richards P: A simple kit for the preparation of ^{99m}Tc-labeled red blood cells. *J Nucl Med* 17: 126–132, 1976

10. Alderson PO, Bernier DR, Ludbrook PA, et al: Serial radionuclide determinations of the ejection fraction with ^{99m}Tc-labeled red blood cells. *Radiology* 119: 729–730, 1976

11. Atkins HL, Eckelman WC, Klopper JF, et al: Vascular imaging with ^{99m}Tc-red blood cells. *Radiology* 106: 357–360, 1973