Labeling of Red Blood Cells with Tc-99m after Oral Administration of $SnCl_2$: Concise Communication

M. C. Patel, P. B. Parab, A. M. Samuel, and R. D. Ganatra

Radiation Medicine Centre, Bombay, India

In vivo labeling of red blood cells with Tc-99m was possible after prior oral administration of $SnCl_2$, both in rats and human volunteers. Absorption of oral $SnCl_2$ was low but sufficient for more than 95% labeling efficiency. Prior i.v. administration of stannous chloride is known to induce in vivo labeling of red blood cells with pertechnetate. We have observed that such labeling is possible even after oral administration of stannous chloride. Nearly 95% of the circulating radioactivity and 93.7% of the administered radioactivity was in RBCs 30 min after i.v. injection of $^{99m}TcO_4^-$ in rats that were fed 5 mg of stannous chloride (3.13 mg Sn^{2+} ion) 2 hr before injection. Red blood cells from four human volunteers could bind pertechnetate, both in vitro and in vivo, after oral administration of 100 mg of $SnCl_2$. We have obtained a blood-pool image of the human heart by labeling the RBCs in vivo by this method. We have also studied various parameters affecting the in vivo binding of RBCs with Tc-99m—such as the amount of orally administered $SnCl_2$, the time of injection of radionuclide after oral $SnCl_2$, and the optimum time for the imaging.

J Nucl Med 20: 877–881, 1979

A number of procedures have been described for the in vitro labeling of red blood cells with [^{99m}Tc] pertechnetate (*1–5*), but none has gained popularity because in all these techniques separation of red blood cells from plasma is required before the labeling. Of late, various investigators have observed in vivo labeling of red blood cells with Tc-99m after prior i.v. administration of stannous ion (*6–11*). Red blood cells labeled in this manner may be useful for blood-pool scanning (*12–14*) or for equilibrium gated blood-pool images for myocardial ejection fraction. Stannous ion in different chemical complexes has been used in varying amounts for this purpose. The optimum conditions of labeling have not been clearly defined. The present study was undertaken to find out whether it was possible to produce in vivo labeling of RBCs after prior oral administration of stannous ion.

MATERIALS AND METHODS

Anhydrous stannous chloride was dissolved in concentrated HCl. This solution was diluted further with normal saline to the requisite concentration (usually 25 mg/ml in 0.05 *N* HCl). For oral administration in clinical studies, the powder was weighed and enclosed in gelatin capsules.

Technetium-99m was obtained from low specific activity Mo-99 by methyl-ethyl-ketone extraction and was finally dissolved in normal saline.

Wistar rats weighing 300–400 g were used for the animal experiments. Animals were divided into three groups: a) those administered varying amounts of $SnCl_2$ orally; b) those to whom an equiv-

Received Apr. 10, 1978; revision accepted Feb. 15, 1979.

For reprints contact: R. Ganatra, Radiation Medicine Centre, % Tata Memorial Hospital, Annexe, Jerbai Wadia Rd., Parel, Bombay-400 012, India.

alent amount of normal saline was given as control; and c) those to whom 5 μg $SnCl_2$ was given intravenously 2 hr before injection of Tc-99m for comparative studies. Two hours after either oral or i.v. administration of $SnCl_2$, 2 ml of blood were collected from the animals by direct heart puncture into 0.5 ml ACD solution to study in vitro binding of Tc-99m to RBCs. The same rats were then injected with 20–25 μCi of pertechnetate in normal saline to study in vivo binding of the Tc-99m to cells. Rats were injected through the penile vein.

Determination of labeling efficiency. Five microcuries of pertechnetate were added to the blood collected as above for in vitro labeling. The mixture was incubated at 37°C for 30 min, and the activity of the whole blood was determined. The blood was then centrifuged, the supernatant removed, and the RBCs washed twice with normal saline. The radioactivities in cells, supernatant, and washings were counted. The activity in cells was expressed as a percentage of total blood activity.

To study in vivo binding of Tc-99m to RBCs, 2 ml of blood were collected in ACD, 30 min after i.v. injection of 20–25 μCi of pertechnetate. Blood was then centrifuged, supernatant removed, and red blood cells washed twice with normal saline. Activity in the cells was expressed as a percentage of the total blood activity.

Organ distribution studies. These were carried out in all three groups of rats described above, 30 min after i.v. injection of pertechnetate. The radioactivity in various organs including blood is expressed as percentage of injected dose. Total blood volume in rats was taken as 7% of the body weight. All possible precautions were taken to maintain adequate geometry during the counting of various organs, and we accounted for 100 ± 10% of the injected activity.

Optimum oral dose of stannous chloride. Four groups of rats (each group containing four animals) were given graded amounts of $SnCl_2$ orally (500 μg, 1 mg, 3 mg, and 5 mg) to find out the dose that gives maximum binding of Tc-99m to RBCs in vitro and in vivo.

To find out how much of the orally administered stannous chloride was absorbed, organ distribution studies were done in a group of four rats after oral administration of 5 μCi of $^{113}SnCl_2$ containing 5 mg carrier. In these experiments only Sn-113 in equilibrium with In-113 was counted.

A group of five rats was fed 50 mg $SnCl_2$ and observed for a period of 3 mo. No untoward effects of this administration were seen. However, elaborate acute or chronic toxicity studies involving blood chemistry, organ histology, etc. were not carried out.

TABLE 1. EFFICIENCY OF BINDING Tc-99m TO RED BLOOD CELLS

	In vitro binding*	In vivo binding*
After 5 mg oral $SnCl_2$	94.92 (92.2–98.70)	96.44 (95.5–97.5)
After 5 μg. i.v. $SnCl_2$	94.8 (92.6–96.3)	94.25 (91.7–96.4)
Controls	23.22 (22.4–23.9)	30.6 (27.8–33.0)

* Figures give % of total activity in blood; mean of four experiments, with range in parentheses.

Human studies. Four volunteers were orally given 200 mg of $SnCl_2$, contained in gelatin capsules. Two hours later, 5 ml of blood were withdrawn for in vitro RBC labeling studies. Five millicuries of pertechnetate were then injected intravenously, and after 30 min the blood-pool images were obtained with a scanner or a gamma camera.

RESULTS

Table 1 shows the efficiencies of in vitro and in vivo binding of Tc-99m to red blood cells in rats, after prior administration of 5 mg $SnCl_2$ orally, 5 μg $SnCl_2$ intravenously, and in controls. The efficiency is virtually the same for in vitro or in vivo binding, irrespective of the route of prior administration of $SnCl_2$. In control animals the binding efficiency was as low as 23–30%. Note that for in vitro labeling of RBCs, prior separation of cells from plasma was not essential.

Table 2 gives the results of organ distribution studies 30 minutes after i.v. injection of 20–25 μCi of pertechnetate in the above three groups of experimental animals. In these, most of the injected radioactivity is in the blood, whereas in controls circulating radioactivity is very low. In the oral group, 93.7% of the injected pertechnetate was in the blood compartment, assuming 7% of the body weight to give total blood volume. In this group, three out of four experimental animals had close to 95% of the injected radioactivity in blood, and only one had 73.5%. Liver, intestine, and carcass all show high activity, probably due to their large blood pools, but the stomach and thyroid show very little activity. As expected, very high concentrations of Tc-99m were observed in thyroid and stomach in control animals.

Table 3 shows the binding efficiency of red blood cells for Tc-99m after prior oral administration of different amounts of $SnCl_2$. With 5 mg of oral $SnCl_2$ (3.13 mg Sn^{2+} ion), the binding efficiencies in vitro and in vivo were 95.1% and 96.8%, respectively.

TABLE 2. ORGAN DISTRIBUTION STUDIES IN RATS, 30 MIN AFTER I.V. INJECTION OF $^{99m}TcO_4^-$. (MEAN OF FOUR RATS; FIGURES IN PARENTHESES SHOW RANGE.)

Organ	5 mg oral $SnCl_2$ 2 hr before $^{99m}TcO_4^-$	5 μg i.v. $SnCl_2$ 30 min before $^{99m}TcO_4^-$	Controls
Blood (2 ml)	7.50 (6.14–9.07)	8.29 (6.47–9.21)	1.69 (1.15–2.27)
Liver	24.30 (19.0–28.3)	24.75 (17.85–31.06)	7.69 (6.88–9.10)
Spleen	0.40 (0.27–0.58)	0.76 (0.45–1.12)	0.085 (0.1–0.18)
Kidneys	3.44 (2.77–4.33)	3.51 (2.85–4.02)	0.79 (0.73–1.49)
Stomach	2.66 (1.76–3.23)	2.52 (1.50–3.27)	14.46 (10.25–16.80)
Intestine	21.33 (13.50–26.63)	21.38 (14.10–25.99)	9.03 (7.28–10.31)
Lungs	1.27 (1.08–1.79)	1.61 (1.42–1.98)	0.91 (0.67–1.13)
Heart	0.30 (0.15–0.59)	0.65 (0.43–0.84)	0.32 (0.19–0.40)
Thyroid	0.39 (0.11–0.53)	0.62 (0.36–0.79)	1.67 (1.26–2.10)
Carcass	42.54 (37.34–54.48)	41.52 (36.89–49.23)	58.04 (51.60–62.31)
Total activity accounted for:	103.74 (99.69–106.47)	105.63 (98.70–113.24)	95.78 (88.95–98.95)
Total blood (7% of body weight)	93.70 (73.5–110.8)	97.75 (83.79–113.55)	20.84 (19.80–22.34)

TABLE 3. EFFICIENCY FOR BINDING OF Tc-99m TO RED BLOOD CELLS, 2 HR AFTER INDICATED ORAL ADMINISTRATION OF $SnCl_2$ (MEAN OF FOUR EXPERIMENTS; FIGURES IN PARENTHESES SHOW RANGE)

$SnCl_2$ dose	In vitro binding	In vivo binding
500 μg	53.9 (47.7–56.7)	60.3 (52.5–73.8)
1.0 mg	59.7 (39.9–84.1)	58.9 (54.1–63.4)
3.0 mg	65.5 (60.03–69.8)	84.7 (79.9–87.9)
5.0 mg	93.1 (88.7–94.5)	96.8 (96.4–97.5)

With smaller amounts of oral $SnCl_2$, the binding efficiencies were low.

Organ distribution studies, carried out 2 hr after oral administration of 5 mg (5 μCi) of $^{113}SnCl_2$, are shown in Table 4. At 2 hr, barely 0.07% of the injected dose was present in the blood. Similarly there was only negligible activity in the liver and kidneys. Most of the activity was not absorbed and appeared to remain in the gut. The results suggest that $SnCl_2$ is poorly absorbed from the gastrointestinal tract. This explains why a large amount of $SnCl_2$ must be administered orally for in vivo RBC labeling.

Fifty milligrams of $SnCl_2$ administered orally in five rats appeared to be well tolerated by the animals without any apparent untoward effects. Similarly all the four volunteers who took 200 mg $SnCl_2$ orally did not report any discomfort.

TABLE 4. ORGAN DISTRIBUTION STUDIES IN RATS 2 HR AFTER 5 mg $^{113}SnCl_2$ BY MOUTH (MEAN OF FOUR RATS; FIGURES IN PARENTHESES SHOW RANGE.)

Organ	% administered dose
Total Blood	0.0675 (0.06–0.08)
Liver	0.12 (0.03–0.23)
Spleen	0.005 (0.0036–0.0075)
Kidneys	0.026 (0.012–0.099)
Stomach	68.53 (58.50–74.99)
Sm. intestine	32.25 (23.54–46.92)
L. intestine	0.91 (0.103–1.78)
Lungs	0.033 (0.009–0.042)
Heart	0.004 (0.001–0.007)
Carcass	0.13 (0.011–0.23)
Total activity accounted for	102.03 (100.43–106.65)

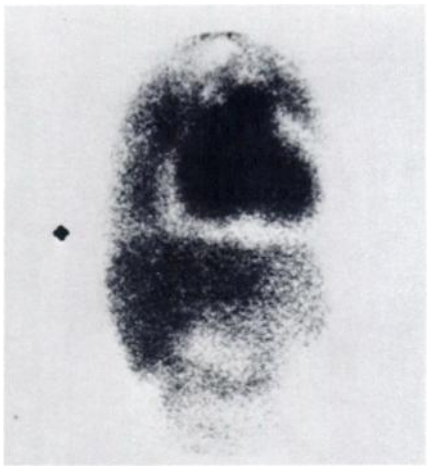

FIG. 1. Blood-pool scintiphoto from a patient with pericardial effusion, 30 min after i.v. injection of 5 mCi $^{99m}TcO_4^-$. Subject was given 200 mg $SnCl_2$ orally 2 hr before injection.

In human experiments the binding efficiencies, both in vitro and in vivo, were over 95% in all subjects (mean 96.65%, range 95.57-97.50).

Figure 1 shows a blood-pool scintiphoto obtained after prior oral administration of $SnCl_2$ in a patient with pericardial effusion. About 5 mCi of $^{99m}TcO_4^-$ were injected in this patient 2 hr after $SnCl_2$.

DISCUSSION

It appears that red blood cells can be labeled with Tc-99m, both in vitro and in vivo with about the same efficiency, after prior oral or i.v. administration of stannous ion. In rats the required i.v. dose was 5 μg, but the oral dose was as high as 5 mg. Smaller amounts administered orally to rats gave lower binding efficiencies. The oral dose required to induce RBC labeling is nearly a thousand times higher because oral stannous ion appears to be poorly absorbed. This was observed in studies carried out in rats with orally administered $^{113}SnCl_2$.

In human trials the oral dose of 200 mg was selected more or less arbitrarily. The dose extrapolated from animal experiments would be 500-600 mg for a 50-kg man, but we thought it prudent to start with the lower dose in our initial experiments. With this dose, we could obtain binding efficiencies that were as high as 95% and 93.7% of the mean percentage administered dose. As Fig. 1 shows, a quite impressive blood-pool image could be obtained with this protocol.

Obviously more work is needed to find out a) whether less than 200 mg would give acceptable labeling efficiencies in humans, and b) whether the waiting period between oral $SnCl_2$ and i.v. pertechnetate could be reduced. We selected a waiting period of 2 hr more or less empirically.

Neither in the animals nor in humans did we observe any untoward effects from our large oral doses of tin. However, more elaborate toxicity studies may be needed if this method is to be used on a routine basis. There are, however, grounds for believing that the toxicity of Sn^{2+} ion, administered orally, would be low. Stannous tin is used in the preservation of foodstuffs and the permissible quantity under Food and Drug Controls in the U.S. is 300 mg/kg (*15*). Tin salts have been mixed with molasses and given orally as a mechanical anthelmintic (*16*). The dose was half an ounce daily for several mornings.

In summary, efficient in vivo labeling of red blood cells is possible by prior oral administration of $SnCl_2$. Since the $SnCl_2$ is given orally, only one injection of pertechnetate is necessary for blood-pool imaging. A patient can swallow a capsule containing $SnCl_2$ 2 hr before coming to the hospital—a procedure akin to the administration of $KClO_4$ before brain scanning.

Since submitting this paper, we have studied 28 patients and obtained excellent blood-pool images after administration of only 100 mg of stannous chloride orally before the injection of pertechnetate.

REFERENCES

1. KORUBIN V, MAISEY MN, MCINTYRE PA: Evaluation of technetium labelled red blood cells for determination of red cell volume in man. *J Nucl Med* 13: 760-762, 1972
2. ECKELMAN WC, REBA RC, ALBERT SN: A rapid simple improved method for the preparation of ^{99m}Tc red blood cells for the determination of red cell volume. *Am J Roentgenol* 118: 861-864, 1973
3. SMITH PH: ^{99m}Tc-labelled erythrocytes for spleen scanning. *Int J Appl Radiat Isot* 25: 137-139, 1974
4. SMITH TD, RICHARDS P: A simple kit for the preparation of ^{99m}Tc-labelled blood cells. *J Nucl Med* 17: 126-132, 1976
5. HARWIG, JF, ALDERSON, PO, PRIMEAU JL: Development and evaluation of a rapid and efficient electrolytic preparation of ^{99m}Tc-labelled red blood cells. *Int J Appl Radiat Isot* 28: 113-121, 1977
6. MCRAE J, SUGAR RM, SHIPLEY B: Alterations in tissue distribution of ^{99m}Tc-pertechnetate in rats given stannous tin. *J Nucl Med* 15: 151-155, 1974
7. CHANDLER WM, SHUCK LD: Abnormal technetium-99m pertechnetate imaging following stannous pyrophosphate bone imaging. *J Nucl Med* 16: 518-519, 1975 (abst)
8. KHENTIGAN A, GARRETT M, LUM D, et al: Effects of prior administration of Sn(II) complexes in vivo distribution of ^{99m}Tc-pertechnetate. *J Nucl Med* 17: 380-384, 1976
9. PAVEL DG, ZIMMER AM, PATTERSON VN: In vivo labelling of red blood cells with ^{99m}Tc: A new approach to blood pool visualisation. *J Nucl Med* 18: 305-308, 1977
10. HAMILTON RG, ALDERSON PO: A comparative evaluation of techniques for rapid and efficient in vivo labelling of red cells with ^{99m}Tc-pertechnetate. *J Nucl Med* 18: 1010-1013, 1977
11. ANCRI D, LONCHAMPT MF, BASSET JY: The effect of tin on the tissue distribution of ^{99m}Tc-sodium pertechnetate. *Ra-*

diology 124: 445-450, 1977

12. ATKINS HL, ECKELMAN WC, KLOPPER JF, et al: Vascular imaging with ^{99m}Tc-red blood cells. *Radiology* 106: 357-360, 1973
13. RYO UY, LEE JI, ZARNOW H, et al: Radionuclide angiography with ^{99m}Tc-labelled red blood cells for detection of aortic aneurysm. *J Nucl Med* 15: 1014-1017, 1974
14. STOKELY EM, PARKEY RW, BONTE FJ, et al: Gated blood pool imaging following ^{99m}Tc stannous pyrophosphate imaging. *Radiology* 120: 433-434, 1976
15. HAMMOND CR: The Elements. In *Handbook of Chemistry and Physics*, 56th ed., Weast RC, ed., Cleveland, CRC Press, 1975, pp B 37-38
16. WOOD HC, LAWALL, YOUNGKEN HW, et al: *The Dispensatory of the United States of America*, Philadelphia, J. B. Lippincott Co., 1937, p 1619

Accepted Articles to Appear in Upcoming Issues

Thyroid Carcinoma in an Autonomously Functioning Nodule (Letter to the Editor). Accepted 2/21/79.
Muhammad Abdel Razzak and James H. Christie

In-Vitro Assessment of Tc-99m-Labeled Bovine Thrombin and Streptokinase-Activated Human Plasmin: Concise Communication. Accepted 3/30/79.
Dennis W. Wong, T. Tanaka, Fred Mishkin, and Timothy Lee

Serial TSH Determination after T_3 Withdrawal or Thyroidectomy in the Therapy of Thyroid Carcinoma. Accepted 3/30/79.
S. V. Hilts, D. Hellman, J. Anderson, J. Woolfenden, J. Van Antwerp, and D. Patton

Noninvasive Estimation of Pulmonary Arterial Pressure by Analysis of Pulmonary Blood-Flow Distribution. Accepted 3/30/79.
Marvin A. Konstam, H. William Strauss, Nathaniel M. Alpert, Stephen W. Miller, Robert X. Murphy, Reginald E. Greene, and Kenneth A. McKusick

A Technique for Measurement of Xenon-133 Air Contamination. Accepted 3/30/79.
Adrian D. LeBlanc, Harlan J. Evans, Philip C. Johnson, and Gerald Timpe

Effects of a Reduction in Crystal Thickness on Anger-Camera Performance. Accepted 3/30/79.
Henry D. Royal, Paul H. Brown, and Ben C. Claunch

Kinetics of the Human Thyroid Trap: Effects of Iodide, Thyrotropin, and Propylthiouracil. Accepted 4/2/79.
Marguerite T. Hays

Effect of Food Intake on the Tissue Distribution of Gallium-67: Concise Communication. Accepted 4/2/79.
Raymond L. Hayes, Janusz J. Szymendera, and Billy L. Byrd

The Bone Scan in Thyroid Cancer (Letter to the Editor). Accepted 4/4/79.
N. David Charkes

Accumulation of Radioiodine in Staff Members (Letter to the Editor). Accepted 4/4/79.
George L. Jackson and Frank MacIntyre

Reply. Accepted 4/4/79.
R. G. Wolfangel

Radioimmunoassay of Free Thyroxine with Prebound Anti-T_4 Microcapsules. Accepted 4/5/79.
Fuad S. Ashkar, Robert J. Buehler, Teresa Chan, and Mokbel Hourani

Moiré Patterns in Gamma Camera Images (Letter to the Editor). Accepted 4/9/79.
R. E. Johnston

Re: Radiation Decomposition of Technetium-99m Radiopharmaceuticals (Letter to the Editor). Accepted 4/12/79.
R. Kishore

Ovarian Imaging by the Binding of Radioiodinated Chorionic Gonadotropin: Concise Communication. Accepted 4/12/79.
C. Y. Lee, S. L. Lee, and Merle K. Loken

Effects of Isoproterenol on Distribution of Perfusion in Embolized Dog Lungs. Accepted 4/12/79.
J. W. Shepard, Jr., D. Hauer, V. Sgroi, and K. M. Moser

Nuclear Medicine and Radionuclide Metrology ICRM Life Sciences Working Group (Letter to the Editor). Accepted 4/12/79.
M. J. Woods

Accumulation of Radioiodinated L-α-Methyltyrosine in Pancreas of Mice: Concise Communication. Accepted 4/13/79.
U. Tisljar, G. Kloster, F. Ritzl, and G. Stöcklin

Evaluation and Application of Alumina-Based Rb-82 Generators Charged with High Levels of Sr-82/85. Accepted 4/17/79.
Yukio Yano, Thomas F. Budinger, Grace Chiang, Harold A. O'Brien, and Patrick M. Grant

Re: Optimization of the Gray Scale for Photoscanners (Letter to the Editor). Accepted 4/18/79.
Frank R. Whitehead

Reply. Accepted 4/18/79.
Wei Chang and Monte Blau

Comparison of Xe-133 Washout and Single-Breath Imaging for the Detection of Ventilation Abnormalities. Accepted 4/19/79.
Philip O. Alderson, Hee Lee, Warren R. Summer, Abbas Motazedi, and Henry N. Wagner, Jr.

A Comparison of Two Technetium-99m-Labeled Radiopharmaceuticals for Lymphoscintigraphy: Concise Communication. Accepted 4/19/79.
William D. Kaplan, Michael A. Davis, and Christopher M. Rose

Constructional Details of Dry Aerosol Delivery System for Scintiphotography of Lungs (Letter to the Editor). Accepted 4/19/79.
P. Kotrappa, B. Raghunath, P. S. S. Subrahmanyam, P. S. Soni, and U. R. Raikar

Radionuclide Left-Ventricular dV/dt and its Dependence on Cardiac Rate (Letter to the Editor). Accepted 4/23/79.
Lawrence E. Williams, Merle K. Loken, Lee A. Forstrom, Richard A. Ponto, and Mathis P. Frick

Re: Tables to Estimate Total Binding Capacity of Thyroxine-Binding Globulin from the In Vivo Thyroid Function Tests (Letter to the Editor). Accepted 5/3/79.
Andries Bakker and Ina Terpstra

Reply. Accepted 5/3/79.
Alfredo Cuarón and Christina Happee de Cuarón