jnm/ PRELIMINARY NOTE

In Vivo Labeling of Red Blood Cells with ^{99m}Tc: A New Approach to Blood Pool Visualization

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A simple and rapid procedure for in vivo labeling of red blood cells (RBC) is presented. The labeling is done with two consecutive intravenous injections, first of "cold" stannous pyrophosphate (Sn-PYP) and then of ^{99m}Tc-pertechnetate. Experimentally, a lag time of 30 min between the two injections was found to result in a mean RBC labeling of greater than 95% during the first hour after pertechnetate injection. A total of 75 patients have been explored by scintillation camera imaging of brain, heart, great vessels, aortic bifurcation, testicles, and lower extremities using the technique outlined. High-quality images were obtained.

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In the past, ^{99m}Tc labeling of red blood cells (RBC) has been used in nuclear medicine for visualization of the blood pool (*1,2*) and the spleen (*3–6*). The labeling methods outlined in the literature involved in vitro methods using stannous chloride, with repeated centrifugations and washing steps before and after addition of ^{99m}Tc-pertechnetate. These steps result in a procedure that is cumbersome, tedious, and time-consuming. More recent in vitro methods of RBC labeling with pertechnetate involve the use of stannous pyrophosphate as the reducing agent (*7,8*). These recent methods have increased the labeling efficiencies and have also decreased the total number of manipulations required in the labeling sequence. In addition, some labeling procedures (*8,9*) are using closed systems so that sterility is not compromised throughout the procedure.

We have considered the possibility of a new approach in labeling RBCs involving an in vivo technique without any manipulation of the red blood cells. The labeling is done by the sequential intravenous injections of "cold" stannous pyrophosphate (Sn-PYP) followed by ^{99m}Tc-pertechnetate.

MATERIALS AND METHODS

Combination in vivo and in vitro study. The purpose of this phase was to determine an appropriate lag time between the injections of stannous pyrophosphate and pertechnetate, and also to determine the RBC-labeling efficiency and the chemical state of ^{99m}Tc in the plasma fraction. Four volunteers each received an intravenous injection of stannous pyrophosphate* (0.2 mg/kg), which had been reconstituted with 5.0 ml of 0.9% NaCl (bacteriostatic free) before injection. At selected intervals after this injection (30, 45, 60, and 120 min), 3-ml blood samples were obtained from each volunteer and added to evacuated glass tubes containing sodium heparin. Two milliliters of whole blood were then incubated with 0.5 ml (approximately 100 μCi) of pertechnetate for 5 min. After the incubation, a whole-blood aliquot was drawn into capillary tubes, sealed, and centrifuged at 4,000 rpm for 5 min. The red-cell fraction was separated from the plasma fraction, the activity of each fraction was measured and corrected for trapped plasma, and the ^{99m}Tc-RBC labeling determined. Chromatograms were run on the plasma fraction using silica-gel instant thin-layer chromatography (ITLC-SG) with acetone and normal saline as solvents. This chromatographic technique distinguishes between pertechnetate, ^{99m}Tc-Sn-PYP, and reduced ^{99m}Tc bound to other chemical moieties.

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* TechneScan PYP, Mallinckrodt Nuclear.

TABLE 1. PROCEDURAL OUTLINE FOR CLINICAL STUDY

1. Intravenous injection of stannous pyrophosphate (0.2 mg/kg body wt., max. injection volume 5 ml, corresponding to a total of 15.4 mg).
2. Approximately 30 min later, 15–20 mCi of ^{99m}Tc-pertechnetate, intravenously.
3. Immediate radionuclide angiogram obtained (sequential images of the area studied).
4. Static images of organs or areas studied.
5. One-minute image of bladder at 30 min after pertechnetate injection.

In vivo study. This study was performed in order to determine the in vivo RBC labeling efficiency when pertechnetate was administered 30 min after intravenous injection of stannous pyrophosphate. In addition, the stability of ^{99m}Tc-labeled red blood cells was determined.

Selected volunteers were injected with stannous pyrophosphate (0.2 mg/kg), which was reconstituted with 5.0 ml of 0.9% bacteriostatic-free NaCl. After a lag time of 30 min, 15–20 mCi of pertechnetate was injected intravenously. At various intervals up to 60 min after the pertechnetate injection, blood samples (approximately 3 ml) were withdrawn and added to sterile evacuated tubes containing sodium heparin. After thorough mixing, 1-ml whole-blood aliquots were delivered into plastic test tubes and the activity was measured and corrected for radioactive decay. The degree of red cell labeling was determined after correction for trapped plasma as described above.

Clinical study. The clinical procedure used for this study is outlined in Table 1 and has been performed on approximately 75 patients. All sequential and static images were obtained with a scintillation camera on line with a computer. After the sequential study over the specific area of interest was performed, static images were obtained (250K to 1M counts) without moving the patient. Brain, heart, great vessels, aortic bifurcation, testicles, and lower extremities were studied. In addition, different angles were used if needed and neighboring or distant areas were also screened. For additional quality control, a 1-min bladder image was obtained in some patients at 30 min after the pertechnetate injection.

RESULTS

The results for the combination in vivo and in vitro study are shown in Figs. 1A and 1B. Figure 1A relates the red-cell labeling yield to the lag time between in vivo stannous pyrophosphate injection and in vitro pertechnetate addition. As the graph indicates, the highest mean red-cell labeling (88%) was achieved with a 30-min lag time. With longer lag times, the experimental labeling decreased only slightly. The chemical state of ^{99m}Tc in the plasma fraction is shown in Fig. 1B. Bear in mind that the total activity in plasma is very small compared with the total activity in the vascular system. With a 30-min lag time, the plasma activity was approximately equally divided between ^{99m}Tc-Sn-PYP and pertechnetate. As the lag time increased, the proportion of ^{99m}Tc-Sn-PYP decreased and that of pertechnetate increased, due to the progressive clearance of pyrophosphate from the vascular pool.

The results of the in vivo study are expressed in Figs. 2A and 2B. Figure 2A shows the in vivo Tc-RBC labeling efficiency up to 60 min after ^{99m}Tc injection. A mean red-cell labeling of 96% was achieved 5 min after injection, and the labeling remained greater than 95% up to 60 min later. The stability of in-vivo-labeled Tc-RBCs is shown in

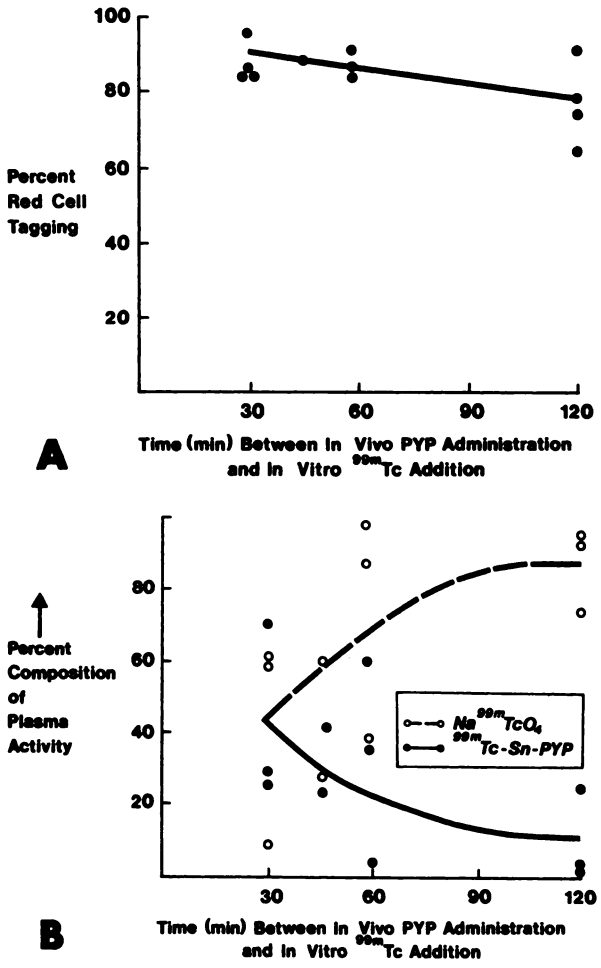

FIG. 1. Combination in vivo and in vitro study. (A) In vitro labeling efficiency as function of lag time between intravenous SnPYP administration and pertechnetate addition, in vitro. (B) Chemical ^{99m}Tc in remaining plasma activity.

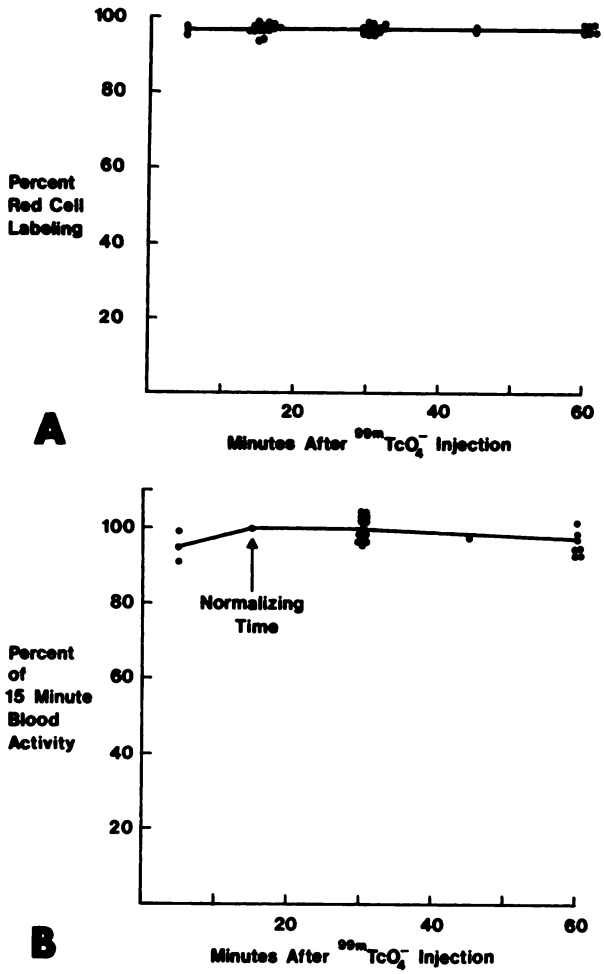

FIG. 2. In vivo study over 60-min interval. (A) Tc-RBC labeling efficiency. (B) Stability of Tc-labeled RBCs.

Fig. 2B. All data obtained were normalized to the 15-min time sample. As the graph indicates, the highest peripheral blood concentration was achieved 15–30 min after pertechnetate injection, and about 95% of this maximum normalized activity was within the vascular pool at 1 hr after injection, indicating the stability of the Tc-RBC complex.

Examples of normal static blood-pool images are given in Figs. 3 and 4; the vascular pools are clearly delineated.

DISCUSSION

In comparison with other ^{99m}Tc-Sn chelates, ^{99m}Tc-Sn-PYP is a weaker complex for which dissociation occurs as exhibited on Sephadex G-25 gel chromatography columns (*10*). In addition, recent investigators (*11–13*) have suggested that red-cell labeling possibly occurred when pertechnetate was injected into patients who had received ^{99m}Tc-Sn-PYP for bone scans in preceding days. This was thought to be due to a complexing of reduced tin with red blood cells and subsequent labeling after pertechnetate injection. Previous studies (*7,8*) have shown in vitro red-cell labeling using Sn-PYP and ^{99m}Tc-pertechnetate. The same labeling phenomenon may occur when "cold" Sn-PYP is previously injected into patients. A stannous RBC complex results, and the excess Sn-PYP is progressively cleared from the vascular pool. When pertechnetate is then injected intravenously, Tc-RBC labeling results.

We have been able to prove that efficient RBC labeling is possible with the sequential technique outlined above. A lag time of 30 min was chosen, based on the dual consideration that this time interval yielded slightly better red cell labeling than longer intervals and also because 30 min is a very convenient interval for the technologists in busy nuclear medicine departments. Very short time intervals of 5–10 min have not been considered due to the impossibility of thorough blood mixing in some patients in less than 10–15 min. Three-hour and 24-hr lag times have also been attempted occasionally, and good blood-pool images have been obtained. In these cases, however, no red-cell labeling studies were performed.

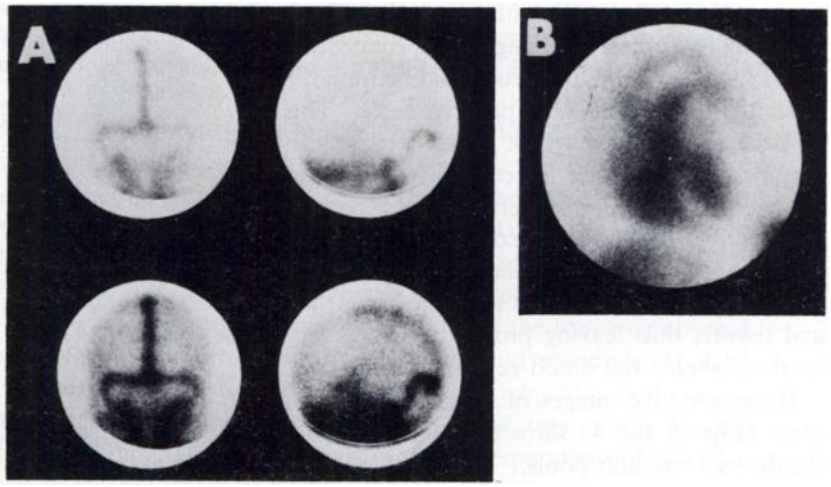

FIG. 3. (A) Normal brain blood-pool imaging. Posterior and left lateral views (two exposures) taken 2 hr after injection. (B) Normal LAO view of heart taken 25 min after injection.

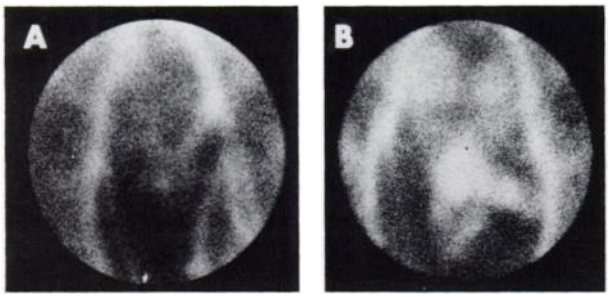

FIG. 4. Scintigrams of pelvic, inguinal, and genital areas (A: female; B: male) taken 15–30 min after injection.

The RBC-labeling efficiency, followed over a 60-min interval, has shown overall a very high degree of labeling (Fig. 2A). In addition, preliminary results indicate that the labeling remains high up to 8 hr after injection. The total amount of activity (corrected for decay) decreased slightly with time (Fig. 2B), indicating a slow rate of leaching of ^{99m}Tc from red blood cells. The bladder, monitored at 30 min, showed a variable degree of radioactivity. In a very few cases, a higher amount of bladder activity seemed to indicate a lower red-cell labeling efficiency. In only two cases, however, was such a correlation established.

A practical problem encountered with this technique is that subsequent partial RBC labeling can occur if pertechnetate is administered during the days following a blood pool scan. Our preliminary studies show a virtual disappearance of RBC labeling with pertechnetate within 7–10 days after the in vivo RBC labeling procedure. In any case, if a brain scan must be performed during this interval, ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) can be used.

For organ imaging we have used time intervals from 3 min to 3 hr after injection with excellent results. The widest range of intervals has been used in brain blood-pool scans. In most of them, when early images were taken (minutes after injection) and compared with delayed images (approximately 2 hr later), no essential differences could be found. Sometimes the delayed images appeared better. This may be because mixing may not be complete during the very first minutes after injection, in which case relatively more free pertechnetate would be available and could diffuse into the extracellular spaces of the scalp and muscles. In delayed images, the small amount of leached ^{99m}Tc is removed from the blood and tissues, thus leaving proportionally a very high level of labeled red blood cells in the vascular pool.

Representative images of brain, heart, and pelvic areas (Figs. 3 and 4) show the very good outline of the desired vascular pools.

The labeling method described adds the capability of in vivo labeling to the already numerous uses of ^{99m}Tc-pertechnetate. While the simplicity and reliability of the method outlined seem well proven, the exact mechanism involved in the RBC labeling is not clear. We are currently investigating this mechanism as well as the minimum concentration of "cold" Sn-PYP needed for adequate RBC labeling. A comparison between in-vivo-labeled Tc-RBCs and ^{99m}Tc-human serum albumin is also under study.

In conclusion, a simple and efficient in vivo method for labeling red blood cells with ^{99m}Tc has been developed. The technique allows the use of high ^{99m}Tc activity, does not require in vitro manipulations, and is very convenient for routine use. The technique outlined marks the beginning of a new use of ^{99m}Tc: in vivo labeling.

ACKNOWLEDGMENT

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