PRELIMINARY NOTE

Use of Technetium-99m as a Radioactive Label to Study Migratory Patterns of Leukocytes

Tatsumi Uchida, Toshio Nemoto, Tokuo Yui, Shin Matsuda, and Shigeo Kariyone

Fukushima Medical College, Fukushima, Japan

Technetium-99m has been used as a radioactive label to study the migratory patterns of neutrophils. In a previous in vitro study, neutrophils were labeled with Tc-99m and infused into patients with and without various hematological disorders. Increased pulmonary localization was detected by scintillation camera within 10 min; this decreased gradually within 3 hr. Accumulation was seen in the liver and spleen at 3 hr. The same results were noted by using neutrophils labeled with Tc-99m sulfur colloid. In a patient with severe ulceration in the oral cavity, due to acute leukemia, Tc-99m-labeled transfused neutrophils that were collected by filtration leukopheresis were concentrated in the infected lesions.

J Nucl Med 20: 1197–1200, 1979

As a white-cell label, Tc-99m should be useful for studying leukocyte kinetics in vivo, with reference to organ distribution of cells. We previously reported in vitro studies of leukocyte labeling with Tc-99m (*1*). Briefly summarized, the labeled leukocytes are viable by the trypan blue exclusion test and phagocytosis index. Elution of Tc-99m from leukocytes suspended in acid citrate dextrose (ACD) is comparable with the elution of tracer from cells labeled with [^{32}P]DFP. Elution of Tc-99m is greater, however, from leukocytes suspended in plasma. Granulocytes label more avidly with Tc-99m than do monocytes, lymphocytes, erythrocytes, or platelets. In the light of these findings, Tc-99m was used in the study of leukocytes in vivo. These results are presented here.

MATERIALS AND METHODS

Eleven patients with various hematological disorders were studied. Their diagnoses and hematological data are given in Table 1. They were informed about the procedure and consent was obtained.

The previously reported technique (*1*) for labeling neutrophils from whole blood was used with the following modifications. The separated leukocytes, resuspended in 10 ml saline containing 2–4 mCi of Tc-99m, were incubated for 10 min at room temperature. This was followed by further incubation (15 min) with 0.3 μg of stannous chloride (0.5 ml of solution from a vial of TCK-11,* consisting of stannous pyrophosphate, sodium pyrophosphate, and sodium hydroxide, dissolved in 10 ml of 0.9% sodium chloride). The leukocyte suspension was then washed twice with physiologic saline, resuspended in 20 ml of saline, and infused intravenously into the patient. Administered doses were 100–500 μCi. Although a commercial kit was used instead of the original 100 μg of $SnCl_2 \cdot 2H_2O$, percentage of labeling efficiency with the new procedure, originally expressed as 100%, was better (140.9 ± 0.7% s.d. for five determinations); and pre-equilibration with stannous solution followed by addition of pertechnetate was 154.3 ± 2.4% (n = 5) in comparison with the original method (100 ± 2.5%, n = 5). In Case 8, Table 1, since RBC contamination was present in the incubation mixture, 50 ml of 0.87% NH_4Cl were added after dextran sedimentation,

Received March 12, 1979; revision accepted May 25, 1979.

For reprints contact: Dr. Tatsumi Uchida, The First Dept. of Internal Medicine, Fukushima Medical College, 4-45 Sugizuma-cho, Fukushima, 960, Japan.

TABLE 1. HEMATOLOGICAL DATA ON PATIENTS INVESTIGATED AND ORGAN DISTRIBUTION OF Tc-99m-LABELED LEUKOCYTES‡

Patient No.	Age (yr) and sex	Diagnosis	WBC/ μl	% of neutrophil series	Liver (cm below costal margin)	Spleen (cm below costal margin)	Migratory patterns of Tc-99m-labeled leukocytes: 10 min after infusion lungs	10 min after infusion liver	10 min after infusion spleen	3 hr after infusion lungs	3 hr after infusion liver	3 hr after infusion spleen
1	40 M	Normal	8,200	67	0	0	+++	+	+	+	+++	+++
2	45 M	Leukocytosis	14,750	78	0	0	ND	ND	ND	+	++	+++
3	68 M	PV	32,000	92	3	8	++	+++	+++	++	+++	+++
4	65 F	CLL	23,300	23	4	*	+++	+	*	+	+++	*
5	34 M	CML	210,000	100	1	4	ND	ND	ND	—	++	+++
6	33 F	CML	31,700	83	2	15	+++	+++	+++	++	+++	+++
7	47 F	CML	177,500	96	2	5	++	+	—	—	+++	++
8	44 M	CML	289,000	95	2	14	+	++	+	—	+++	+++
9	45 F	CML	260,000	94	3	7	++	++	—	—	+++	+
10	33 M	CML	320,000	98	3	10	++	++	+	—	+++	++
11	37 F	AL	1,700	10	1	0	++	+	+++	+	+	+++

‡ Abbreviations used: PV = polycythemia vera, CLL = chronic lymphocytic leukemia, CML = chronic myelocytic leukemia, AL = acute leukemia, * = after splenectomy, +++ = marked uptake, ++ = moderate uptake, + = weak uptake, ND = not determined.

and the RBCs were hemolysed.

In Cases 2 and 5, simultaneous leukocyte labeling with Tc-99m (2–4 mCi) and Cr-51 (300 μCi) was performed. The Cr-51 labeling technique is described by Scott et al. (*2*). The leukocytes from serial timed venous blood samples were separated by the method previously reported (*3*), and Cr-51 radioactivity was expressed as counts per min per 10 million leukocytes. The expected leukocyte specific activity and the proportion of labeled leukocytes remaining in the circulation after infusion, which is called "recovery," were calculated by the method of Mauer et al. (*4*).

The reliability of this method was checked by comparison with migratory patterns of leukocytes labeled with Tc-99m sulfur colloid (*5,6*) (Cases 9 and 10).

In Case 11—a patient with severe ulceration of the oral cavity, gingivitis, high fever, and neutropenia resulting from chemotherapy of acute leukemia—neutrophil transfusion was performed by filtration leukopheresis (FL). From a final volume of 300 ml containing 15×10^9 neutrophils, 20 ml of cell suspension were labeled with Tc-99m as before, and labeled cells were infused into the patient.

The gamma scintillation camera was used for immediate imaging of Tc-99m-labeled leukocytes. Scintiphotographs of the whole body were made until 4 hr after the completion of the infusion.

RESULTS

Scintigrams were performed on 11 patients with various hematological disorders. A scintigram of the normal lungs was positive within 10 min, and the liver and spleen had the highest uptake at 3 hr after the infusion. Figure 1 (A, B) and Table 1 show the organ distributions at 10 min and 3 hr after the infusion of leukocytes labeled with Tc-99m. The lungs were highly radioactive within 10 min, but the activity decreased gradually within the next 3 hr, by which time there was much radioactivity in the spleen and liver. Uptake was most pronounced in patients with polycythemia vera and chronic myelocytic leukemia. Serial camera images up to 3 hr showed no accumulation in the thyroid gland, stomach, kidneys, or bone marrow.

The pulmonary uptake during the early period was studied further. Scintigrams of the lungs were made every 2 min until 10 min after the infusion; radioactivity in that area reached a maximum in 2 to 4 min, and decreased thereafter.

The results of simultaneous labeling with Tc-99m and Cr-51 in Cases 2 and 5 show that the recovery of infused cells was 31.3% and 45.1%, respectively, in Case 2, and 24.9% and 30.0% in Case 5 (with splenomegaly).

Phagocytic leukocytes labeled by incubating blood cells in vitro with Tc-99m sulfur colloid were used for comparison with Tc-99m-labeled white cells (Fig. 1C). Migratory patterns of neutrophils carrying phagocytized Tc-99m sulfur colloid were the same as for those tagged with Tc-99m.

Figure 1D shows the scintiphotograph made 3 hr after the infusion of neutrophils Tc-99m-labeled by FL; the labeled neutrophils accumulated in the infected area.

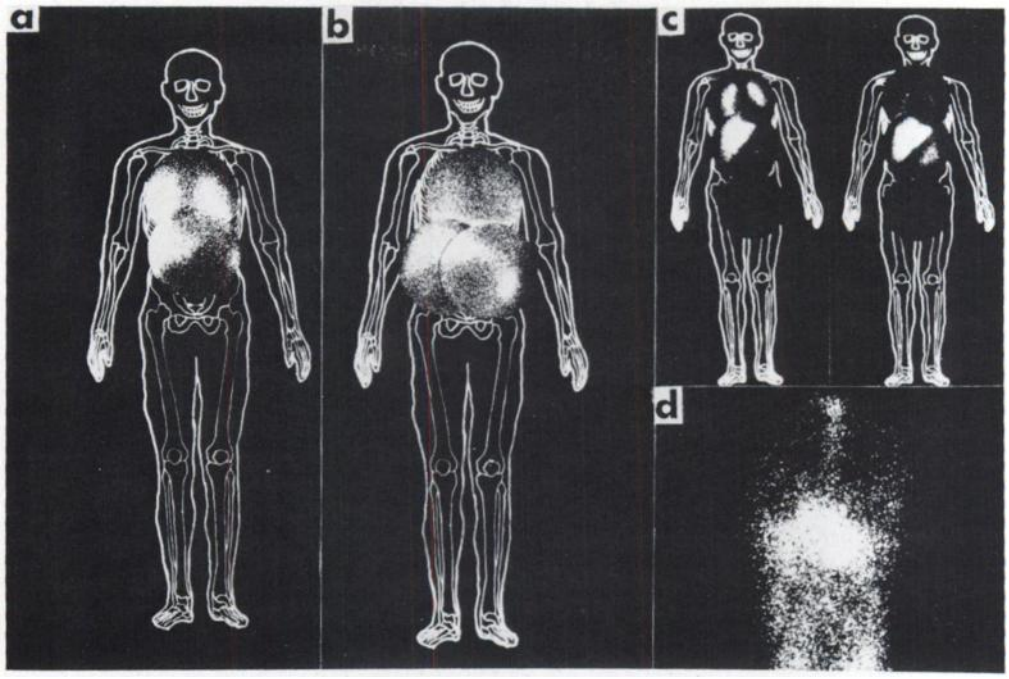

FIG. 1. (A,B). Migratory patterns of Tc-99m-labeled leukocytes 10 min (A) and 3 hr (B) after infusion in normal subject. In (A) note accumulation of radioactivity in lungs, and rather poor uptake in liver and spleen. Figure B shows marked uptake in liver and spleen, and poor uptake in lungs. (C) Migratory patterns of neutrophils carrying phagocytized Tc-99m sulfur colloid (Case 9). Ten minutes after infusion, labeled neutrophils were trapped in lungs; thereafter released gradually. Uptake in liver and spleen was noted 3 hr after infusion. These results were the same as with Tc-99m-labeled white cells. (D) Neutrophils Tc-99m-labeled by leukopheresis accumulated in inflammatory lesions of acute leukemia patient with gingival ulcers (Case 11).

DISCUSSION

The previous report showed that neutrophils can be labeled in vitro with Tc-99m. The great usefulness of this method is likely to be in the study of cell migration and organ uptake, as monitored by external scanning. The migration pattern of Tc-99m-labeled leukocytes was evaluated in a normal subject and in patients with various hematological disorders. Uptake was marked in lungs, liver, and spleen. Other investigations, using [^{32}P]DFP-labeled granulocytes, show two granulocyte pools (circulating and marginal pools), between which granulocytes equilibrate freely (*7,8*). We have found no published information concerning the organ distribution of the marginal pool, and our results suggest that lungs, spleen, and liver contain most of the marginal granulocyte pool.

The lower immediate vascular recovery obtained with Tc-99m-labeled leukocytes observed in studies using (Tc-99m and Cr-51) doubly-labeled leukocytes (Cases 2 and 5) may be due to elution of Tc-99m in plasma as we reported previously (*1*).

An alternative explanation for prominent uptake by lungs in the early phase might be damage or aggregation of cells during the labeling procedure. No gross aggregation, or dead cells monitored by trypan blue exclusion, was noted by light microscopy. The other labeling technique—that of permitting neutrophils to phagocytize Tc-99m sulfur colloid particles in vitro, as documented by English et al. (*5,6*)—was also used, and the same migratory pattern of neutrophils was found. In this method, however, there seems to be some problems during the separation of labeled cells from the original colloidal particles. These migratory patterns also appeared with autologous leukocytes labeled with In-III oxine (Thakur et al., *9*) in which there was initial accumulation of radioactivity in the lungs; approximately half of this cleared in 15 min and 25 to 50% of radioactivity distributed in liver and spleen (*9,10*). These findings might suggest that neutrophils were trapped in the pulmonary vasculature as a marginal pool.

FOOTNOTE

* CIS, France.

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