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Intravascular survival of leukemic cells labeled with Indium-111-Oxine

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A method for labeling leukemic cells *in vitro* with ^{111}In-oxine is described. Intravascular survival data and organ distribution of ^{111}In-oxine-labeled leukemic cells in patients with acute leukemia are presented. No evidence of diminished cell viability or significant elution of the label could be found by the *in vitro* studies. Disappearance curves of ^{111}In-labeled leukemic cell radioactivity in the circulation were a single exponential with average T1/2 value of 15.3±2.5 hr (mean±SEM), which was found to be prolonged when compared with the results of 4 hematologically normal subjects (7.0±0.8) and those of previously reported DF^{32}P studies. Migratory patterns of the labeled leukemic cells, obtained by a scintillation camera, demonstrated sequestration in the lungs 5 min after the infusion, and thereafter, the uptake into the spleen and liver gradually increased. We believe that the properties of ^{111}In-oxine might overcome many of the difficulties of studying leukemic cell kinetics with cells labeled with tritiated thymidine.

Key words: Leukokinetics, Leukemia, In-111-oxine

INTRODUCTION

IT IS WELL KNOWN that leukemic cells divide more slowly and take longer to synthesize DNA than normal hematopoietic precursors, and acute leukemia is not due to rapid cellular proliferation.[1,2] However, leukemic cells accumulate in various organs in most patients. In the peripheral blood, the ^{3}H-TdR labeling index of leukemic cells is lower than in the bone marrow.[3] When the transit times have been calculated by following the disappearance from the blood of the heavily labeled cells, values from 33 to 36 hr have been obtained.[4,5] Liquid scintillation studies of peripheral blood radioactivity after the injection of ^{3}H-TdR in relapse show 2 peaks of radioactivity,[6,7] which return to a normal pattern with remission. However, leukemic cell kinetic studies in the peripheral blood are not performed widely, because ^{3}H-TdR or ^{3}H-cytidine are long half-life radioisotopes.

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In 1976, ^{111}In-oxine was introduced as a blood cell label,[8] and subsequently cellular kinetic studies have been performed by using ^{111}In-oxine-labeled neutrophils,[9] lymphocytes,[10] and platelets.[11] Recently, using ^{111}In-oxine we were able to label leukemic cells[12] and as a consequence have been able to perform kinetic studies on peripheral leukemic cells of different leukemic status. We examined the survival and pool sizes of ^{111}In-oxine-labeled leukemic cells in acute leukemia and the results are presented here.

MATERIAL AND METHODS

In vitro studies of leukemic cell labeling with ^{111}In-oxine were examined. Leukemic cell incubation, labeling efficiency, label elution by repeated washes and incubation for 10 hr, viability by trypan-blue supravital staining[13] and a leukemic colony forming unit (L-CFU)[14] with or without ^{111}In were evaluated *in vitro*. The *in vitro* colony technique with leukemic cells is the use of phytohemagglutinin as a stimulator in the preparation of a leukocyte-conditioned medium (PHA-LCM). After the removal of T-lymphocyte precursors by the E-rosette formation, labeled or non-labeled leukemic cells were cultured and the

Table 1 Hematologic data in patients with acute leukemia and blastic crisis of chronic myelocytic leukemia

Case No.	Age/Sex	Diagnosis	Leukocytes (/μl)	% of leukemic cells	Number of cells injected ($\times 10^7$)	% of leukemic cells injected
Normal Values (mean±SEM)			7,575±144			
1	58/M	AML (M2)	9,300	57	3	78
2	82/M	AML (M2)	90,650	52	47	74
3	34/M	AML (M2)	123,900	86	108	86
4	57/M	AML (M2)	53,900	95	51	100
5	30/F	AML (M2)	6,000	47	3	64
6	49/M	AML (M2)	23,800	83	19	96
7	40/M	AMMoL(M4)	30,700	66	20	93
8	72/F	AMMoL(M4)	3,500	54	1	62
9	62/M	AMoL (M5b)	7,700	79	6	89
10	51/F	AMoL (M5b)	29,900	83	24	98
11	36/F	BC of CML	45,600	84	38	90
12	14/M	BC of CML	88,700	20	18	69
13	46/F	ALL (L1)	4,600	87	4	100

AML: acute myelogenous leukemia, AMMoL: acute myelomonocytic leukemia, AMoL: acute monocytic leukemia, ALL: acute lymphocytic leukemia, BC of CML: blastic crisis of chronic myelocitic leukemia.

numbers of L-CFU, erythroid colony forming unit (CFU-E) and colony forming unit in culture (CFU-C) were counted.

Thirteen patients with acute leukemia were studied. According to the declaration of Helsinki,[15] all the procedures were fully explained to the patients, who gave their consent. The patients' peripheral leukocyte and leukemic cell counts that were investigated are shown in Table 1. Fifty to two hundred milliliters of heparinized whole blood (5 unit heparin/m*l* blood) were collected from each patient in a sealed sterile plastic bag, which was centrifuged at 1,000 g for 8 min. A buffy coat was obtained and diluted 2 to 3 times by physiological saline. The leukemic cells were separated from the buffy coat by the Ficoll-Hypaque gradient separation method.[16] The leukocyte-rich plasma was layered onto a Ficoll-Hypaque density gradient. The gradient tubes were centrifuged at 1,500 g for 40 min, at which time the leukemic cells and platelets were concentrated at the interface between the Ficoll-Hypaque solution and plasma. Leukemic cells and platelets were separated by centrifugation at 150 g for 10 min, with the leukemic cells at the bottom. The washed cells were resuspended in 5 m*l* of physiological saline without albumin. Labeling with ^{111}In-oxine was carried out at room temperature for 20 min. The radioactivity used was between 200–500 μCi and the labeled leukemic cells were washed 3 times in a physiological saline solution or autologous plasma at 300 g for 5 min. The final labeled cells were resuspended in 10 m*l* of autologous plasma and was infused into the patient. The differential cell counts are shown in Table 1. Ten milliliters of blood was withdrawn from each patient 5, 30, 60, 180, 300 minutes and 1, 2, 3 days after the administration of ^{111}In-oxine-labeled leukemic cells to the patients. Leukemic cells were separated by the method described above. The cell numbers were counted by an electric cell counter (Sysmex E-2000). The radioactivity of the separated cells was measured with an automatic well-type scintillation counter. A minimum of 5,000 counts was obtained for each sample by integral counting with a background of 7 to 9 cpm. The radioactivity of the leukemic cells had a wide variation from 20 to 100 cpm according to the leukemic cell counts in the peripheral blood. Disappearance curves of the labeled cells were obtained by plotting the radioactivity against time. The labeled cells decreased in a single exponential fashion, and half-life (T1/2) was calculated. As a result of the isotope dilution principle, the size of total blood leukemic cell pool (TBLCP) can be determined.[17,18]

$$\text{TBLCP}=\frac{\text{Radioactivity of leukemic cells injected} \times \text{number of leukemic cells injected}}{\text{Radioactivity of blood leukemic cells at } t_0}$$

From the leukemic cell counts and the blood volume, the number of leukemic cells in the circulation, which is defined as circulating leukemic cell pool (CLCP), can be calculated. The marginal leukemic cell pool (MLCP) can be calculated by subtracting the CLCP

from the TBLCP. The proportion of labeled cells remaining in the systemic circulation after infusion, i.e., "% recovery" was calculated from the radioactivity per milliliter extrapolated to zero time, multiplied by the estimated blood volume, and divided by the ^{111}In activity injected. The leukemic cell turnover rate (LCTR), defined as the number of leukemic cells turned over through the blood each day per kg body weight, can also be calculated.

RESULTS

In vitro studies

Incubation time. The labeling efficiency, i.e., the ratio of the radioactivity of the labeled leukemic cells to that of the ^{111}In utilized was 58.9±0.7% within 10 min.

Label elution. Repeated washings of labeled leukemic cells were performed to measure the elution of the label. The loss of radioactivity from the labeled leukemic cells as a result of each wash was not significant after one wash (after 2nd washing: 67.9±0.7%, after 6th washing: 66.6±1.0%). A sample of ^{111}In-labeled suspension was incubated at 37°C for 10 hr. Serial specimens were taken and the radioactivity of the supernatant and the cell viability by trypan-blue supravital staining[13] were determined. This indicated that significant label elution from intact leukemic cells did not occur and a decrease in the radioactivity depended on cell damage or lysis during *in vitro* incubation (loss of radioactivity at 3 and 6 hours: 3.1±0.8, 7.4±1.0%; % of viability at 3 and 6 hours: 98.4±1.1%, 93.0±1.5%, respectively).

Viability. The effect of the labeling procedure on leukemic cell viability was evaluated using two methods. Repeated studies of preinfusion suspensions by supravital staining with trypan blue showed that less than 5% of the cells were stained immediately after preparation.[13]

The ability of *in vitro* colony formation in ^{111}In-labeled and non-labeled leukemic cells was compared using 6 patients. There were no differences in CFU-C (82 colonies without In: 90 with In, 58 without In: 57 with In), CFU-E (361 colonies without In: 349 with In) or L-CFU numbers (678, 86, 581 colonies without In: 680, 55, 434 colonies with In, respectively).

In vivo studies

Neutrophil disappearance curves labeled with ^{111}In-oxine in 4 normal individuals showed an exponential decrease with a half-life of 7.1±0.8 (mean±SEM) hours (leukocyte counts: 7,575±287/μl, neutrophil counts: 4,474±1,190/μl). Disappearance curves of ^{111}In-oxine labeled leukemic cells are shown in Fig. 1.

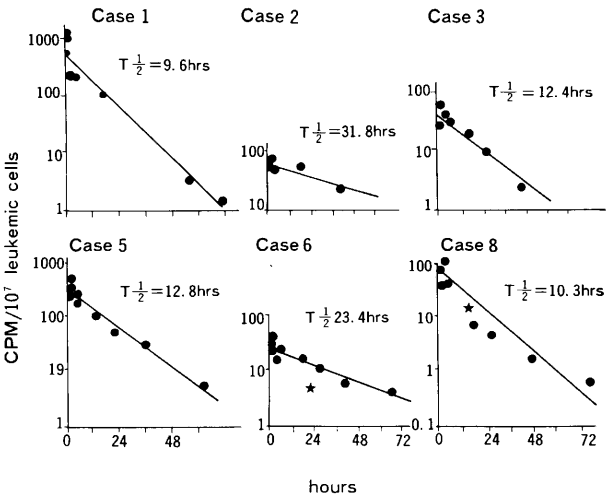

Fig. 1 Leukemic cell disappearance curve in patients with acute leukemia. The curves show the single exponential decrease and that the half-life of the disappearance was prolonged.

A single exponential decrease is also shown in all cases. T½ of leukemic cells is shown in Table 2, ranging from 3.5 to 31.8 hr at 15.1±7.5 hr, which is longer than that of normal neutrophils labeled with DF^{32}P,[15,18] and ^{111}In-oxine.[19] Leukemic cell pool sizes are extremely enlarged (Table 2). The ratio of CLCP: TBLCP was 0.32±0.08. LCTR is also larger than granulocyte turnover rate[19,20] and there is no relationship between LCTR and TBLCP.

As a result of an observation with a scintillation camera, it was shown that labeled leukemic cells accumulated chiefly in the spleen and liver in all cases (Fig. 2). Accumulation in the lungs was seen a few minutes after the injection and decreased thereafter. The bone marrow was visualized in 4 cases. In case 4, with a cutaneous involvement of leukemic cells, labeled leukemic cells were seen in the skin; but in case 13, a tumor-forming leukemic patient, no labeled cells were seen in the tumor tissues.

Leukemic cell survival in 2 patients with blastic crisis of chromic myelogenous leukemia was measured. T½ of leukemic blasts was 13.0 hr (38,300/μl of blasts) and 16.8 hr (18,100/μl of blasts), respectively. Leukemic cell kinetic patterns were similar to those of acute leukemia.

DISCUSSION

The results of these investigations indicate that ^{111}In-oxine is an effective label for human leukemic cells in peripheral blood. The uptake of the label was efficient, and the label did not appear in the supernatant after repeated washings of ^{111}In-labeled leu-

Table 2 Cell kinetic data in patients with acute leukemia and blastic crisis of chronic myelocytic leukemia

Case No.	Age/ Sex	Disappearance rate T 1/2 (hours)	Intravascular recovery (%)	Pool size indices				Remarks
				TBLCP ($\times 10^7$/kg)	CLCP ($\times 10^7$/kg)	MLCP ($\times 10^7$/kg)	LCTR ($\times 10^7$/kg/day)	
Normal Values (mean±SEM)		7.1±0.8						
1	58/M	9.6*	28.4	81	23	58	140	before treatment
2	82/M	31.8*	31.1	1,131	352	779	591	before treatment
3	34/M	12.4*	36.6	2,080	762	1,318	2,785	before treatment
4	57/M	25.6*	21.7	1,683	365	1,328	1,097	before treatment
5	30/F	12.8*	21.0	100	21	79	131	relapse
6	49/M	23.4*	45.1	295	133	162	210	relapse
7	40/M	11.1*	33.7	407	137	270	608	before treatment
8	72/F	10.3*	2.6	314	8	306	506	relapse
9	62/M	13.1*	16.1	274	44	230	347	before treatment
10	51/F	—†	—	—	159	—	—	before treatment
11	36/F	13.0*	40.0	642	257	385	824	blastic crisis
12	14/M	16.8*	20.4	608	124	484	603	blastic crisis
13	46/F	3.5*	3.9	381	15	366	1,793	before treatment

* single exponential decrease, † random destruction

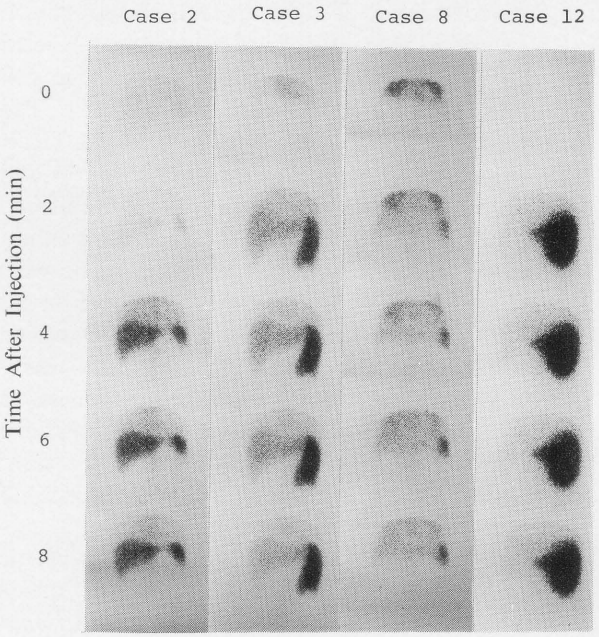

Fig. 2 Organ distribution of ^{111}In-oxine labeled leukemic cells in cases 2, 3, 8 and 12. Labeled leukemic cells accumulated chiefly in the spleen and liver in all cases.

kemic cells *in vitro*. It was also demonstrated that the ^{111}In-oxine complex was not reutilizable and spontaneous release of ^{111}In-oxine from tumor cells was every low.[19]

Cell viability was assessed by a trypan-blue exclusion test and *in vitro* colony assay of leukemic cells. Leukemic cell progenitors in the peripheral blood of acute myeloblastic leukemia form colonies when stimulated by a leukocyte-conditioned medium (PHA-LCM). In order to obtain blast cell colonies, the technique in which T-lymphocyte colonies were removed by forming rosettes with sheep erythrocytes was developed.[14] We evaluated the leukemic cell viability with or without ^{111}In-oxine by forming leukemic cell colonies. There was no difference in the leukemic colonies with or without ^{111}In-oxine.

Labeled leukemic cells when returned to the circulation equilibrate between two blood pools as do normal granulocytes. There are the circulating and the marginal pools.[17,18] Disappearance curves of ^{111}In-oxine-labeled leukemic cells showed a single exponential fashion in all cases investigated, which is similar to the normal neutrophil decrease.[18,21,22] In leukemic cells labeled with ^{3}H-TdR or 3H-UdR, T½ of leukemic cells is 23,[2] 25,[4] and 28 hr,[23] respectively. The sojourn time of leukemic cells in peripheral blood has been determined only in a few cases because recently the use of labeled DNA precursors has been prohibited in human beings. Since then, no suitable radionuclides for leukemic cell labeling have been determined. ^{111}In-oxine labeled leukemic cells would overcome this restriction.

Survival of ^{111}In-oxine labeled leukemic cells was prolonged in comparison with ^{111}In-oxine-labeled normal neutrophils (5.0±1.6 hr of T½)[22] and DF^{32}P-labeled neutrophils (6.7±1.2 hr,[18] 9.9±2.7 hr)[21]; however, were shortened in comparison with T½ in

patients with chronic myelocytic leukemia.[24,25] A recent study demonstrated that the clearance curve of radioactivity showed a plateau or the appearance of a hump from 1 to 5 hr after the injection of ^{111}In-labeled leukemic cells.[26] This radioactivity was expressed by whole blood gamma counting and leukemic cells were not separated. In a steady-state condition, however, the reappearance of random labeled leukemic cells as a "hump" in the circulation could not be experienced.

Enlarged TBLCP was demonstrated, and the ratio of CLCP to TBLCP was smaller than in the entire normal group 0.44,[18] which would suggest that leukemic cells easily migrate in the marginal pool and infiltrate into various organs.

Another advantage of ^{111}In-oxine-labeled leukemic cell kinetics is that it is possible to utilize the scintigraphic technique to determine the migration patterns of leukemic cells which do not circulate. These images indicate that the liver and spleen are the major organs of uptake of ^{111}In-radioactivity. This probably represents margination and infiltration of leukemic cells into these organs. Organ distribution of normal individuals revealed accumulation in the spleen (9–22%) and liver (12–14%).[27] The reversible removal of leukemic cells was observed in the lungs. Bone marrow activity appeared in some cases with acute leukemia and was less intense in normal individuals. In one case with cutaneous involvement of leukemic cells, labeled leukemic cells accumulated in the skin.

In patients with chronic myelocytic leukemia, the disappearance rate of DF^{32}P-labeled leukocytes is much slower than in normal people[24,25]. The reason for this is probably due to the presence of immature cells in the blood and the grossly expanded total blood granulocyte pool. Other studies suggested that immature granulocytes in chronic myelocytic leukemia were capable of migrating in the bone marrow, from the blood of the spleen and from the spleen to the marrow,[23,28] which might be the cause of the long granulocyte survival. In patients with acute leukemia such a cellular traffic as occurs in chronic leukemia might not be demonstrated because leukemic cells do not survive very long.

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