PRELIMINARY NOTE

Cell Damage Resulting from the Labeling of Rat Lymphocytes and HeLa S3 Cells with In-111 Oxine

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Rat thoracic-duct lymphocytes and HeLa S3 cells were labeled in vitro with different amounts of indium-111 oxine. The labeled rat lymphocytes were tested for their ability to recirculate normally in syngeneic rats; the labeled HeLa S3 cells for their ability to divide to form colonies in tissue culture. Both cell types behaved normally by these criteria when labeled with small amounts of indium-111 oxine but at higher doses were obviously damaged. Evidence was obtained for the HeLa S3 cells that this damage was primarily radiation-induced. These findings may impose limitations on the use of In-111 oxine as a cell label for clinical purposes.

J Nucl Med 20: 1308–1311, 1979

Indium-111 oxine has been shown to be of use as a marker for polymorphonuclear leukocytes (*1*) and lymphocytes (*2*) in a variety of clinical studies. For these studies it is important that the labeled cells should remain viable and functionally normal. Damage could occur during the separation of the cells from whole blood and from the treatment of the cells with In-111 oxine. Both radiation and chemical damage to the cells might be expected because of the predicted radiation dose from the low-energy Auger electrons emitted by the In-111 (D. J. Sylvester, personal communication) and the known and suspected toxicity of at least some of the contaminants found in In-111 oxine preparations.

Evidence of cell damage resulting from In-111 oxine cell labeling was sought by measuring the ability of labeled rat thoracic-duct lymphocytes to recirculate normally and by assaying the colony-forming ability of labeled cultured human tumor cells (HeLa S3) as a measure of mitotic cell health.

MATERIALS AND METHODS

A pure population of lymphocytes was obtained by cannulation of the thoracic duct in inbred rats. The lymphocytes were suspended in phosphate-buffered saline at 10^8 cells/ml and incubated at room temperature for 15 min with different amounts of nominally carrier-free In-111 oxine in ethanol prepared by the method of Thakur et al. (*3*). The cells were then washed twice in saline by low-speed centrifugation, and once in fetal calf serum in order to remove excess In-111 oxine, which binds to serum transferrin. Viability after labeling as assessed by trypan blue dye exclusion was greater than 95%. The percentage of In-111 incorporated in the cells was consistently 85–95%. Following labeling, the lymphocytes were injected intravenously into syngeneic rats. All rats received the same number of cells. Recipients were killed 2 or 24 hr after injection and the tissues were removed, weighed, and the In-111 measured. The amount of activity present in different tissues was expressed as a percentage of the injected radioactivity per gram of tissue.

Mitotic arrest of HeLa S3 cells was determined by measuring their in vitro colony-forming ability after labeling with In-111 oxine. The cells were labeled at 2×10^5 cells/ml in saline for 15 min at room temperature and then diluted 1:8000 in tissue-culture medium containing 15% fetal calf serum. Two hundred cells in tissue culture medium were plated into 5-cm petri dishes and incubated undisturbed for 10 days. During this time the viable cells attached to the dishes and divided in situ to produce visible colonies, which were stained and counted.

Received March 6, 1979; revision accepted June 13, 1979.

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TABLE 1. THE DISTRIBUTION OF In-111-LABELED THORACIC-DUCT LYMPHOCYTES IN SYNGENEIC RATS

Experiment	μCi In-111 oxine per 10^8 lymphocytes	Tissue	Percentage injected radioactivity per gram of tissue*	
			2 hr after injection	24 hr after injection
1	5	Lymph nodes	1.2 (0.9–1.6)	15.2 (12.5–17.2)
		Spleen	54.0 (49.4–56.3)	41.4 (38.9–42.7)
		Liver	2.4 (2.1–2.7)	2.4 (1.5–3.1)
	150	Lymph nodes	2.0 (1.7–2.4)	3.1 (2.5–3.6)
		Spleen	75.1 (71.4–80.1)	77.2 (74.2–79.6)
		Liver	3.3 (3.1–3.7)	4.7 (4.2–5.5)
2	5	Lymph nodes	1.3 (1.2–1.5)	24.2 (22.9–25.1)
		Spleen	40.0 (35.1–43.4)	32.1 (29.0–35.4)
		Liver	0.8 (0.6–1.0)	1.0 (0.9–1.1)
	150	Lymph nodes	8.6 (8.3–8.9)	9.4 (9.1–9.9)
		Spleen	83.5 (83.1–84.2)	65.0 (64.2–66.2)
		Liver	2.3 (1.9–2.5)	1.4 (1.1–1.6)

* Six rats were injected with each cell population. The figure given at each time period is the mean and range of tissues from three animals.

RESULTS

Table 1 presents the results of two experiments that examined the capacity of lymphocytes labeled with In-111 oxine to migrate to lymphoid tissue. Lymphocytes labeled with 5 μCi In-111 oxine showed behavior similar to that of lymphocytes labeled with conventional radiotracers in other studies, i.e., there was a 15- to 20-fold increase in the amount of activity in the lymph nodes between 2 and 24 hr, and the activity in the spleen was high at 2 hr but dropped significantly at 24 hr. These findings reflect the fact that the bulk of lymphocytes injected intravenously initially enter the spleen. A proportion of the cells leave the blood in the spleen and migrate through the tissue with a transit time of several

TABLE 2. THE DISTRIBUTION IN SYNGENEIC RATS OF LYMPHOCYTES LABELED WITH DIFFERENT AMOUNTS OF In-111 OXINE

μCi In-111 oxine per 10^8 lymphocytes*	Tissue	Percentage injected radioactivity per gram of tissue†	
		2 hr after injection	24 hr after injection
5	Lymph nodes	6.7 (6.4–7.0)	27.0 (24.7–29.3)
	Spleen	61.0 (58.8–63.2)	36.6 (35.4–37.8)
10	Lymph nodes	4.3 (3.9–4.7)	31.0 (29.8–32.2)
	Spleen	52.0 (51.1–52.9)	43.7 (42.6–44.8)
20	Lymph nodes	4.8 (4.7–4.9)	35.0 (32.4–37.6)
	Spleen	56.8 (52.5–61.1)	35.1 (33.6–36.6)
40	Lymph nodes	4.8 (4.5–5.1)	14.0 (13.3–14.7)
	Spleen	44.0 (42.4–45.6)	24.0 (21.9–26.1)
80	Lymph nodes	4.9 (4.3–5.5)	3.6 (3.1–4.1)
	Spleen	64.2 (62.4–66.0)	66.4 (61.9–70.9)

* The percentage In-111 incorporated in the cells was 86.3 ± 2.4% (mean ± s.d.).
† Data obtained in a single experiment. Four rats were injected with each cell population. The figure given at each time period is the mean and range of tissues from two animals.

hours. Cells that do not leave the blood in the spleen will be available to the lymph nodes. By 24 hr a steady state has been reached in which the labeled lymphocytes are distributed throughout the recirculating pool and are therefore present in significant numbers in both the spleen and lymph nodes.

In contrast, when cells were labeled with 150 μCi In-111 oxine there was little fall in the activity in the spleen between 2 and 24 hr and very little lymph-node localization at 24 hr. The increased level of activity in both the spleen and liver is compatible with sequestration of dead or damaged cells. These findings were confirmed by injecting the same cell populations intravenously into rats with thoracic-duct fistulae; the cells labeled with 5 μCi migrated from blood to lymph normally, whereas the cells labeled with 150 μCi failed to appear in thoracic-duct lymph.

The relationship between the amount of In-111 bound and the ability of lymphocytes to migrate to lymphoid tissue was examined more closely in experiments in which lymphocytes were labeled with several different doses of In-111 oxine. The results of one such experiment (Table 2) showed that cells labeled with up to 20 μCi In-111 behaved normally. At higher doses of In-111 oxine, migration of the cells to lymph nodes was significantly impaired. Other experiments suggested that only at 5 μCi In-111 oxine per 10^8 cells do lymphocytes consistently behave normally: labeling with 10–40 μCi produced aberrations in tissue distribution that varied from slight to severe, while lymphocytes labeled with 100–150 μCi always failed to migrate normally.

The effect of In-111 oxine on the human tumor cell

TABLE 3. COLONY-FORMING ABILITY OF HeLa CELLS LABELED WITH In-111 OXINE

Experiment*	In-111 oxine μCi/ml	μCi bound per 2 × 10^5 cells	Percentage binding	Colony-forming ability (% control)†	Colony-forming ability (% control) using decayed In-111 oxine‡
1	1.17	0.09	8	100	
	2.0	0.196	10	100	
	10.0	2.86	29	73	
	22.5	6.42	29	2.8	
2	0.56	0.076	14	91	
	1.1	0.17	15	73	
	4.9	1.98	40	11	
	10.5	5.46	52	0.1	
	22.4	13.72	61	0.01	94
3	13.3	0.24	2	61.5	
	28.5	0.86	3	11.0	
	59.7	4.94	8	0.86	100
4	5.1	0.54	11	99	
	10.6	1.38	13	96	
	22.4	2.18	10	57	83
5	8.4	0.30	4	98	
	17.0	0.80	5	89	
	30.4	1.37	5	15	80
6	1.05	0.09	9	100	
	5.1	0.71	14	100	
	10.2	1.65	16	100	
	20.0	3.7	18	33.5	
	25.4	4.5	18	4.0	73

* Each experiment used a different batch of $^{111}InCl_3$.
† Unlabeled HeLa S3 cells.
‡ Data obtained from a single experiment in which cells were labeled with In-111 oxine freshly prepared from each batch of decayed $^{111}InCl_3$.

line HeLa S3 was determined by testing the ability of labeled cells to divide to form colonies in tissue culture. The results of several experiments (Table 3) showed that the colony-forming ability of HeLa cells was reduced after labeling with In-111 oxine, and that the reduction was dependent on the amount of In-111 oxine used to label the cells. Inhibition of colony-forming ability correlated more closely with the amount of radioactivity to which the cells were exposed than with the amount bound to the cells. There was, however, considerable variation between experiments in both the percentage binding of In-111 oxine and in the effect on colony-forming ability for a given amount of In-111 oxine bound. In each experiment a different batch of $^{111}InCl_3$ was used to prepare the In-111 oxine. A partial explanation for the observed variation may have been the presence of different amounts of stable metal impurities, especially cadmium, in the $^{111}InCl_3$ batches used. These would complex with the excess oxine used in the preparation of the In-111 oxine, forming other toxic metal-oxine complexes.

Damage to cells after labeling with In-111 oxine might also be caused by radiation from the decay of In-111. In order to determine to what extent radiation toxicity was responsible for the reduced colony-forming ability of HeLa cells treated with In-111 oxine, the same batches of $^{111}InCl_3$ that were used previously were left to decay and then used to prepare fresh solutions of "indium-oxine." When cells were treated with an amount of the decayed preparation equivalent to the maximum concentration of In-111 oxine previously tested, the percentage colony-forming ability was greater than 70% in all cases (see Table 3). This finding suggested that radiation from the decay of In-111 was a major cause of damage to the cells.

DISCUSSION

Although the two test systems used are quite different, they nevertheless show that labeling of either cell type with In-111 oxine could result in impaired function. These findings are in agreement with the recent demonstration of impaired function of both lymphocytes and neutrophils labeled with In-111 oxine (*4*). In the experiments reported here, the damaging level of In-111 binding per cell was less than or similar to the amounts currently being used to label leukocytes for abscess localization in patients, since it is necessary to use at least 100 μCi In-111 in order to obtain satisfactory external scanning. Consequently it is very important that the possibility of damage to cells by In-111 oxine should be investigated for each cell type being considered for clinical tracer studies. Work is in progress to examine different means and conditions of labeling cells with In-111, by which damage to the cells may be minimized.

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