Toxicity of Indium-111 on the Radiolabeled Lymphocyte

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The radiolabeling of lymphocytes with ^{111}In has resulted in detectable toxic changes in the cells. The mechanisms of toxicity for lymphocytes have been related to the label's radioactivity and to the chelator used to mediate the intracellular localization. These mechanisms were examined by assessing cellular function with mitogen-mediated blastogenesis after labeling lymphocytes with either the chelator (tropolone) alone, ^{111}In complexed with tropolone, or cadmium (the decay product of ^{111}In) complexed with tropolone. Successful lymphocyte labeling with ^{111}In was shown to be dependent upon the concentration of the chelator (tropolone). Increasing concentrations of tropolone inhibited lymphocyte function to a variable degree. Further reduction in cellular function was detected after incorporation of a constant amount of ^{111}In or ^{111}In's decay product, cadmium. Lymphocyte function was decreased by these two labels in a parallel linear manner. This same toxic effect was seen after labeling with small constant amounts of tropolone and increasing quantities of ^{111}In or cadmium. Thus, although both the required chelator and the radiobiologic exposure have a deleterious effect on the lymphocyte, significant lymphocyte toxicity appears to result from the metal-to-cell interaction as a result of the metal decay product (cadmium).

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Radiolabeling of hematopoietic cells poses a variety of diagnostic and therapeutic opportunities. To date, the moiety most commonly studied is indium-111 (^{111}In) linked to a chelate, generally oxine or tropolone. Indium-labeled granulocytes (*1,2*) and platelets (*3,4*) provide an established in vivo method for obtaining diagnostic information. Unfortunately, lymphocyte labeling has met with varied success. Moreover, the [^{111}In]-chelator complex adversely affects lymphocyte function (*5–7*). The toxic effects that have been described include defects in the lymphocytic "proliferative capacity" (mitogen-mediated blastogenesis) (*5,6*) and a variety of chromosomal changes after radiolabeling (*6*). Although oxine at high concentrations has been shown to affect lymphocyte function (*5*), the current view is that [^{111}In]-chelator complex as it exists has detrimental effects as a direct result of ionizing radiation (*5–7*).

Recent studies from this laboratory have focused on lymphocyte structure and function when labeled with ^{111}In; most of these have used the chelate tropolone for labeling. The present studies found that the toxicity to the lymphocyte may be due not only to radiation, but also to the chemical properties of cadmium, the decay product of ^{111}In.

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MATERIALS AND METHODS

Lymphocyte Isolation

Blood was obtained from volunteers by venipuncture into a heparin containing syringe. The lymphocyte fraction was isolated with Ficoll-Paque* as previously described (*8*). This separation procedure results in a fraction containing >90% viable lymphocytes. The viability was corroborated by trypan blue exclusion. The isolated cells were washed three times and then diluted in Roswell Park Memorial Institute (RPMI) 1640 media.†

Chelate Preparation

Tropolone‡ (2-hydroxy-2,4,6-cyclo-heptatrienone) was prepared under sterile conditions at a concentration of 4.4 m*M* in hepes saline buffer with the final pH adjusted to 7.4 with 0.1 N NaOH.

Radionuclide and Cadmium Preparation

Carrier-free ^{111}In§ in 0.05*M* HCl was used as the radionuclide in all studies. The radionuclidic purity was assayed to be 99% with an activity of 2 mCi on the calibration date.

Indium-111 was diluted with 0.05*M* HCl to an adequate working volume for cellular labeling.

An equimolar concentration (3.6 n*M*) of cadmium chloride ($CdCl_2$)[¶] was prepared with 0.05*M* HCl as the solvent.

Labeling Lymphocytes with [^{111}In]Tropolone or Cadmium-Tropolone

The isolated lymphocytes were prepared for labeling in RPMI 1640 media at a concentration of 1 × 10^6 cells/ml of media. The cells were then incubated with a range of concentrations of tropolone (5–25 μg/10^6 cells) and either an increasing amount of ^{111}In (0.1–5 μCi/10^6 cells) or an equal volume of the 3.6 n*M* solution of cadmium chloride. The incubation time was limited to 10–15 min. Following incubation, the cells were washed twice with RPMI. Quantitation of radionuclide incorporation was determined after the labeling and washing procedure. Measurement of the amount of radiolabel incorporated was done with a gamma counter.[**]

In Vitro Studies of Lymphocyte Function

After labeling, the lymphocytes were placed in culture media (RPMI 1640 + 10% fetal calf serum + 1% penicillin/streptomycin); the final concentration was 1 × 10^6 cells/ml of culture media. Lymphocyte function was assessed by measuring the activity of mitogen mediated blastogenesis with tritiated thymidine uptake. The mitogen used was phytohemagglutinin P[††] at 1 μl of 1% solution per 10^6 cells.

The labeled cells in culture were evenly distributed in 200 μl fractions on 96-well plates[‡‡] and incubated in the presence of mitogen at 37°C in an atmosphere of 5% CO_2 for 48–60 hours. Blastogenesis was then quantitated by measuring radioactive DNA incorporation of tritiated thymidine after a 12-hr pulse (1 μCi per 200 μl well). Lymphocyte function was evaluated in this way by 6–15 separate in vitro studies.

Statistical Analyses

All data is reported as mean values ± s.d. unless otherwise stated. Results were assessed by one factor analysis of variance and by Student-Neuman Keuls Multiple Comparison Testing to make appropriate pairsome comparisons. Significance was determined by a critical value of p = 0.05.

RESULTS

Cellular Incorporation to [^{111}In]Tropolone

The typical radiolabeling curve developed with incorporation of [^{111}In]tropolone into lymphocytes as shown in Figure 1. Indium-111 labeling was directly related to the concentration of available chelator, again confirming the requirement for chelator mediated transport into the lymphocyte. Of note is the labeling of 54.8% (2.74 μCi) of available ^{111}In (5 μCi/10^6 cells) at the tropolone concentration of 15 μg/10^6 lymphocytes and the lack of significant further radiolabeling with higher amounts of tropolone.

Lymphocyte Function Following [^{111}In]Tropolone Labeling or Cadmium-Tropolone Labeling

Normal lymphocytes were radiolabeled with a constant amount of indium-111 (5 μCi/10^6 cells) chelated

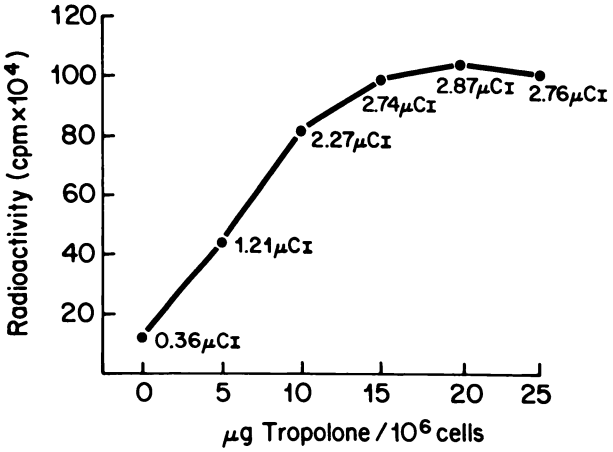

FIGURE 1
Concentration of intracellular ^{111}In relative to amount of available tropolone. The lymphocyte incorporation of the ^{111}In is recorded on the curve (amount of ^{111}In/10^6 cells) which plots detected radioactivity in counts per minute (cpm × 10^4) against the concentration of tropolone. The total amount of ^{111}In available for labeling was 5 μCi/10^6 cells.

serially to increasing concentrations of tropolone (5–25 μg/10^6 cells). After labeling, lymphocyte function was determined by measurement of tritiated thymidine uptake after mitogen (PHA) induced blastogenesis (Fig. 2). As a control, blastogenesis was measured by lymphocytes "labeled" in buffer alone.

Figure 2 is a representative example of lymphocyte function evaluation after labeling with either cadmium-tropolone or [^{111}In]tropolone by three determinations. As depicted in Figure 2, a decrease in blastogenesis was observed after lymphocytes were "labeled" with only tropolone. The addition of ^{111}In to complete the radiolabel produced a more pronounced suppression of blastogenesis than that seen when the cells were exposed to tropolone alone.

Equimolar concentrations of cadmium (the decay product of ^{111}In) and tropolone were complexed and used as a cell label. The labeling followed the identical methods used in [^{111}In]tropolone labeling. Again, blastogenesis was impaired as the tropolone to cell concentration increased (5–25 μg/10^6 lymphocytes) (Fig. 2). The cadmium-tropolone complex produced a more toxic effect when compared with lymphocytes labeled with only tropolone. The toxicity of cadmium-tropolone coincided with the damage produced by cells labeled with ^{111}In. This affect on lymphocyte function depended on the presence of tropolone since cells incubated and tested with ^{111}In or cadmium alone (in the absence of tropolone) showed negligible functional change.

Lymphocyte function was also examined using smaller amounts of ^{111}In or cadmium and a constant concentration of tropolone (Table 1). Blastogenesis with

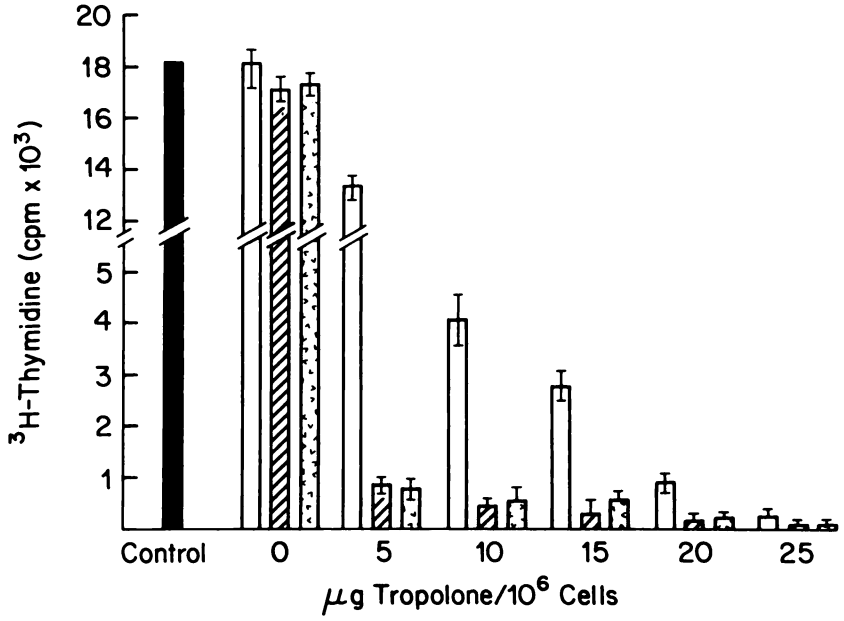

FIGURE 2
Comparative mitogenic (phytohemagglutinin P) response of normal lymphocytes after a 15-min incubation with either increasing concentrations of tropolone alone (□), increasing concentrations of tropolone chelated with a constant amount of $CdCl_2$ (3.6 n*M* solution) (▨), or increasing concentrations of tropolone chelated with a constant amount of ^{111}In (5 μCi/10^6 cells) (▩). Control panel (■) represents cells cultured in the presence of Hepes Buffer and 0.05*M* HCl alone. ^{3}H-thymidine uptake is expressed as cpm (cpm × 10^3).

tropolone (5 μg/10^6 cells) and doses of ^{111}In (0.1–1.0 μCi/10^6 cells) or equimolar amounts of cadmium were not significantly different from mitogenesis seen with tropolone (5 μg/10^6 cells) only (Table 1). Statistically significant ($p = 0.05$) toxicity (additional to that seen with just tropolone) was observed with the smaller amounts of either ^{111}In or cadmium when the tropolone concentration was increased to 10 μg/10^6 cells (Table 1).

TABLE 1
Hydrogen-3 Thymidine Uptake by Phytohemagglutinin Stimulated Lymphocytes after Labeling with a Constant Amount of Tropolone and Either Increasing Amounts of ^{111}In or Increasing Equimolar Quantities of Cadmium ($CdCl_2$)*

	^{3}H-thymidine uptake (cpm × 10^3)/10^6 cells† Tropolone/10^6 Cells	
	5 μg	10 μg
Control‡	42.49 ± 5.32	32.63 ± 7.56
^{111}In (0.1 μCi/10^6 cells)	50.60 ± 3.00	16.67 ± 7.70§
$CdCl_2$ (Equimolar)	47.40 ± 2.54	27.52 ± 2.54
^{111}In (0.5 μCi/10^6 cells)	50.30 ± 3.30	20.30 ± 2.00§
$CdCl_2$ (Equimolar)	42.57 ± 1.30	18.70 ± 1.67§
^{111}In (1.0 μCi/10^6 cells)	41.50 ± 10.90	19.44 ± 8.59§
$CdCl_2$ (Equimolar)	42.90 ± 5.70	20.60 ± 3.20§

* All cells were labeled with a 10–15 min exposure to tropolone and ^{111}In or tropolone and cadmium.

† Each result represents the summation of 6 determinations and are expressed as a mean value and standard deviation. cpm = counts per minute.

‡ The control represents the uptake of cells incubated with tropolone only.

§ Significant change ($p = 0.05$) when compared to control cell uptake.

DISCUSSION

Physiologic and pathophysiologic studies of the fate of circulating cells require the experimental method to be free of cellular injury. Thus, any influences on the labeled cell's function limits its usefulness. The search for a valid lymphocyte radiolabel has encountered several problems relating to cellular function. Previous studies have shown that the [^{111}In]oxine complex influences the labeled lymphocyte's "proliferative capacity" (*5,6*) as well as the cell's chromosomal architecture (*6*). It is currently thought that the biologic effect of the indium-chelator complex on the cell is mediated by ionizing radiation emitted by the label (*5,6*).

The present study specifically examined the chemical effects of cadmium (the decay product of ^{111}In) on the proliferative capacity of the lymphocyte. The chelate used to mediate radiolabeling was tropolone. We have previously described tropolone's cytotoxic effects (*9*). These effects parallel those produced by oxine. Like oxine, tropolone can impair lymphocyte blastogenesis and cellular viability (*9*). This effect can be minimized by decreasing the concentration of tropolone (5 μg/10^6 cells) (*9*) as well as the incubation period (10-15 min) during the labeling procedure (*9*).

The incorporation of radioactive indium mediated by tropolone was found to be dependent on the chelator's concentration during labeling. As the amount of tropolone increased, the amount of ^{111}In incorporated also increased with a radiolabeing plateau reached at a tropolone concentration of 15 μg/10^6 cells.

Lymphocyte function was influenced after exposure to tropolone, tropolone plus ^{111}In, or tropolone plus cadmium. As the concentration of tropolone increased, cellular mitogenesis was progressively impaired. In-

dium-111 tropolone suppressed lymphocyte blastogenesis more than that detected after labeling with only tropolone. An identical pattern of mitogenic suppression was found when cells labeled with tropolone were compared with those labeled with the tropolone-cadmium complex. Less mitogenic activity was detected after cellular labeling with tropolone-cadmium as opposed to labeling only with the chelate. Cellular activity after tropolone-cadmium labeling was the same as that detected after labeling with [^{111}In]tropolone. Impaired mitogenesis was even evident with small amounts of ^{111}In (0.1 μCi/10^6 cells) or equimolar amounts of cadmium and depended on the presence of an adequate amount of chelate (10 μg/10^6 cells). Lack of blastogenic inhibition with smaller amounts of chelate complexed with smaller amounts of ^{111}In or cadmium most likely reflect a less efficient cellular incorporation of the complete radiolabel.

Cadmium's ability to disrupt the human chromosome is a well-recognized entity (*10*). Chromosomal analysis on workers in zinc smelting plants (an environment containing zinc, lead, and cadmium dust and fumes) showed an increased amount of chromosomal aberrations (*10*) that would prove (in vitro) to be secondary to cadmium (*11*). In addition, cadmium has been indicted to affect the lymphocyte's ability to produce lymphokines (*12*). The process by which the chromosomal changes occur is unclear, but is most likely related to the intranuclear location of metallothionein (*13*) (a cadmium binding protein). The chromosomal changes produced by cadmium are random with a variety of gaps, accentric fragments, and open chromatid breaks (*10*), not unlike those found after radiolabeling with [^{111}In]oxine (*6*).

It has been estimated that the intra- to extracellular concentration ratio after labeling with the radionuclide complex ([^{111}In]oxine) is >100:1 (*14*). It is also known that the cadmium concentration in the blood of individuals exposed to cadmium (and having chromosomal breaks) is $\sim 2 \times 10^{-7}$ *M* $CdCl_2$ (*10*). It is then reasonable to expect significant lymphocyte damage with the nanomolar concentrations of cadmium used in this study. Even though chromosome changes were not assessed specifically, it can be safely assumed that chromosomal patterns were significantly altered. Ten Berge et al. have proposed that chromosomal changes may precede any measured change in mitogen mediated blastogenesis (*6*).

These findings emphasize and help define the mechanism of lymphocyte toxicity when radiolabeling with the indium-chelator complex. Our findings indicate that the amount of indium incorporated is strictly dependent on the amount of chelate (tropolone) used. In addition, we find that the functional defect of the radiolabeled lymphocyte may be due not only to an effect of ionizing radiation but also to a chemical toxicity from the heavy metals indium and its daughter, cadmium.

NOTES

* Pharmacia Inc., Piscataway, NJ.
† Gibco Labs, Grand Island, NY.
‡ Aldrich Chemical, Milwaukee, WI.
§ DuPont Company, No. Billerica, MA.
¶ Baker Chemical Co., Phillipsburg, NJ.
** (NML-5000) Nuclear Medical Laboratories, Inc., Irving, TX.
†† Difco Laboratories, Inc., Detroit, MI.
‡‡ Data Packaging Corp., Costar Div., Cambridge, MA.

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REFERENCES

1. Peters AM, Severymuttu SH, Reavy HJ, et al. Imaging of inflammation with indium-111 tropolonate labeled leukocytes. *J Nucl Med* 1983; 24: 39–44.
2. Thakur ML, Coleman RE, Welch MJ. Ind-111 labeled leukocytes for localization of abscesses: preparation, analysis, tissue distribution, and comparison with Gallium-67 citrate in dogs. *J Lab Clin Med* 1977; 89: 217–228.
3. Scheffel U, Tsan Min Fu, McIntyre PA. Labeling of human platelets with [^{111}In] 8-hydroxyquinolone. *J Nucl Med* 1979; 20: 524–531.
4. Dewanjee MK, Rao SA, Rosemark JA, et al. Ind-111 tropolone, a new tracer for platelet labeling. *Radiology* 1982; 145: 149–153.
5. Segal AW, Deteix P, Garcia R, et al. In-111 labeling of leukocytes: a detrimental effect on neutrophil and lymphocyte function and an improved method of cell labeling. *J Nucl Med* 1978; 19: 1238–1244.
6. Ten Berge RJM, Natarajan AT, Hardeman MR, et al. Labeling with In-111 has detrimental effects on human lymphocytes: concise communication. *J Nucl Med* 1983; 24: 615–620.
7. Chisholm PM, Danpure HJ, Healey G, et al. Cell damage resulting from the labeling of rat lymphocytes and HELA S3 cells with In-111 oxine. *J Nucl Med* 1979; 20: 1318–1311.
8. Harris R, Ukaejiopa EO. Tissue typing using a routine one step lymphocyte separation technique. *Br J Haematol* 1970; 18: 229–235.
9. Balaban EP, Simon TR, Sheehan RG, et al. The effect of the radiolabel mediator tropolone on lymphocyte structure and function. *J Lab Clin Med* 1986; 107: 306–314.
10. Bauchinger M, Schmid E, Einbrodt HJ, et al. Chromosome aberrations in lymphocytes after occupational exposure to lead and cadmium. *Mutat Res* 1976; 40: 57–62.
11. Gasiorek K, Bauchinger M. Chromosome changes in human lymphocytes after separate and combined

treatment with divalent salts of lead, cadmium, zinc. *Environ Mutagen* 1981; 3: 513–518.
12. Bendtzen K. Induction of antigen specific lymphocyte unresponsiveness in vitro: possible role of divalent cations and defective function of human T-cell-activating factor (TAF). *Cell Immunol* 1982; 66: 152–163.
13. Hildebrand CE, Cram LS. Distribution of cadmium in human blood cultures in low levels of $CdCL_2$. Accumulation of Cd in lymphocytes and preferential binding to metallothionein. *Proc Soc Exp Biol Med* 1979; 161: 438–443.
14. Kassis AI, Adelstein SJ. Chemotoxicity of indium-111 oxine in mammalian cells. *J Nucl Med* 1985; 26: 187–190.