Effect of Unlabeled Indium Oxine and Indium Tropolone on the Function of Isolated Human Lymphocytes

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The purpose of this study was to compare the effect of indium oxine and indium-tropolone complexes (nonradiolabeled) on the function of isolated human lymphocytes. Peripheral lymphocytes were obtained from 15 normal volunteers and incubated with indium oxine or indium tropolone according to the standard techniques currently used when cells are radiolabeled for subsequent in vivo studies. The phytohemagglutinin-induced (PHA) lymphocyte transformation and a more specific lymphocyte functional test (the mixed lymphocyte reaction) were performed following incubation with the indium complexes. The results indicate that PHA transformation is not affected by either indium oxine or indium tropolone, whereas both chelates reduced the mixed lymphocyte reaction. This suggests that these substances have a selective toxic effect only on a functionally distinct lymphocyte subset (i.e., the cytotoxic T cells) and indicates that there is no significant difference between the two indium chelates in terms of their effect on lymphocyte function.

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The labeling of peripheral white cells with indium-111 (^{111}In), using either oxine or tropolone as chelates, is now used frequently when abscesses and other sites of inflammation are localized by scintigraphy (*1–4*). This technique is also applied to the study of organ-specific autoimmune diseases by using purified labeled lymphocytes (*5–7*).

The choice of chelate, to mediate the transport of indium into the cells, is important since cells may be damaged by the complex indium chelate independently of the radiation dose. This reduces both the circulation capacity of the cells and the extent to which they come into the affected organ.

Several studies have been carried out in vitro concerning the viability, the random migration, the chemotaxis, and the bacterial capacity of neutrophils labeled with ^{111}In, using either oxine or tropolone as chelate (*8–10*). But very little information is available on the in vitro effect of indium oxine and/or indium tropolone on lymphocyte function.

The present investigation was therefore undertaken in order to evaluate the in vitro effect of these two complexes (nonradiolabeled) on mitogen-induced lymphocyte transformation and on the mixed lymphocyte reaction.

The aim of the study was to compare the potential damaging effects of the two chelates and, in consequence, to define which is more suitable for in vivo studies using purified lymphocyte preparations.

MATERIALS AND METHODS

Venous blood (20 ml) was collected from 15 normal volunteers (11 M, 4 F—mean age 27 yr). Mononuclear cells were obtained by the centrifugation of blood on Ficoll Hypaque gradient (*11*). Cells were washed twice in phosphate buffer saline (PBS) and counted. After washing, platelet contamination in the cell preparation was minimal.

Preparation of indium tropolone

The indium-tropolone complex was prepared ac-

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cording to the methods suggested by Dewanjee and Dampure (*12,13*). Tropolone was prepared at a concentration of 0.5 mg/ml in Hepes saline buffer containing 20 m*M* Hepes in 0.8% v/v sodium chloride. The indium-tropolone complex was obtained by adding 0.1 μg/ml of indium (as $InCl_3$) which had been previously dissolved in HCl 0.04*M*. The pH of the indium-tropolone solution was 7.4. The tropolone molecules added allow all the indium atoms present in the solution to be complexed. This preparation corresponds to the one used for in vivo studies, except that in this case indium is not radiolabeled.

Preparation of indium oxine

As oxine has been demonstrated to be soluble in an aqueous solution (*14,15*), the chelate was prepared in a saline solution which contained 6 mg/ml of Hepes buffer, at concentration of 50 μg/ml. The solution was prepared at 40° and left for 1 hr in a shaking bath. The indium-oxine complex was obtained by adding 0.1 μg/ml of indium (as $InCl_3$), which had previously been dissolved in HCl 0.04*M*, to the solution. The pH of the indium-oxine solution was 7.2. The solution corresponds to one of the commercially available products used for in vivo studies, except that our solution contains indium, oxine, Hepes, and saline. It does not contain other adjuvants normally included in the commercial products, since these could have additional toxicity. Even in this case, the oxine molecules added allow all the indium atoms present in the solution to be complexed.

Cell cultures

Lymphocytes (1.0×10^7) were preincubated for 7 min at room temperature with 5 μl of indium tropolone in a final volume of 100 μl of autologous plasma. Alternatively, they were preincubated for 20 min at room temperature with 5 μl of indium oxine in a final volume of 200 μl of Hank's balanced salt solution (HBSS). These two procedures correspond to those used for in vivo studies (50 μCi of [^{111}In]tropolone for 10^8 cells in 1 ml of plasma or 50 μCi of [^{111}In]oxine for 10^8 cells in 2 ml of HBSS). Control cultures include lymphocytes which were preincubated with HBSS alone. Following incubation, the lymphocytes were washed twice in HBSS and two sets of cultures were prepared for the following functional tests.

Phytohemagglutinin-induced lymphocyte transformation (PHA transformation)

This was performed according to the method described by Victorino et al. with slight modifications (*16*). Briefly, lymphocytes were resuspended in RMPI 1,640 culture medium + 10% fetal calf serum at a concentration of 1.0×10^6/ml and cultured for 72 hr at 37°C in a 5% CO_2 incubator. Four hours before the end of the culture, [^{125}I]deoxyuridine (0.5 μCi/2.0×10^5 cells) was added and the samples were then counted after they had been washed three times in HBSS.

Mixed lymphocyte reaction (MLR)

This was performed according to the method described by Moretta et al. with slight modifications (*17*). Briefly, target lymphocytes were prepared by incubating the cells for 30 min at 37°C with mitomycin-C and then washing three times. In order to evaluate whether a residual of mitomycin-C could have affected lymphocyte function, target cells were irradiated with 2,000 rad instead of mitomycin-C in some experiments. No difference was observed between the two methods in the final results. Effector lymphocytes (cells preincubated with indium complexes) were resuspended in RPMI 1,640 culture medium + 10% fetal calf serum at a concentration of 1.0×10^6/ml and cultured for 5 days at 37°C in a 5% CO_2 incubator. The effector:target ratio was 1:1. Four hours before the end of the culture, [^{125}I]deoxyuridine (0.5 μCi/2.0×10^5 cells) was added and the samples were then counted after they had been washed three times in HBSS.

RESULTS AND DISCUSSION

As shown in Table 1, PHA-induced lymphocyte transformation was not affected by either indium tropolone or indium oxine. Mixed lymphocyte reaction was significantly reduced when lymphocytes were preincubated with indium oxine ($p < 0.01$) and a tendency towards a reduction of MLR was also observed with lymphocytes treated with indium tropolone (Table 2). The fact that only a more specific T cell function (MLR) is impaired by oxine suggests that this chelate has a selective toxic effect on some lymphocytes only (i.e., cytotoxic T cells).

However, when the phenotypes of different functional subsets of lymphocytes, including cytotoxic T cells in resting stage, were analyzed using the same doses of indium oxine (even if radiolabeled), no variation of the phenotypic expression of cytotoxic T cells was noted; nor was the cell viability affected (*18*). Our data suggest that the indium chelates impair the specific function of only a small percentage of well differentiated and functionally distinct lymphocytes. However, since the fraction of added chelates which remain intracellular following incubation and washing is unknown, it is not possible to determine the precise mechanism of cell damage induced by the chelates. As regards the in vivo studies using indium-labeled lymphocytes, we think it likely that this toxic effect is not of great importance since cytotoxic T cells represent only a small proportion of total lymphocytes and their reduction does not prohibit their migration capacity; the latter can be better investigated by means of kinetic studies in vivo.

TABLE 1
The Effect of Indium Tropolone and Indium Oxine on the PHA-Induced Lymphocyte Transformation

Case no.	Lymphocytes preincubated with medium alone*	Lymphocytes preincubated with indium tropolone*	Lymphocytes preincubated with indium oxine*
1	4.42	4.53	4.54
2	4.55	4.62	4.38
3	4.64	4.70	4.58
4	4.34	4.48	4.26
5	3.87	3.84	3.86
6	4.24	4.21	4.11
7	4.19	4.27	4.29
8	4.89	4.84	4.80
9	4.21	4.09	4.01
10	4.15	4.28	4.22
11	4.24	4.28	4.32
12	4.68	4.83	4.77
13	4.63	4.70	4.71
14	4.59	4.65	4.63
15	4.76	4.79	4.77
Mean ± s.d.	4.43 ± 0.27	4.47 ± 0.30	4.42 ± 0.30
Paired t-test†		N.S.	N.S.

* Values are expressed as logarithmic convertion of ^{125}I deoxyuridine uptake by lymphocytes.

† Paired t-test was calculated compared with lymphocytes preincubated with medium alone.

TABLE 2
Effect of Indium Tropolone and Indium Oxine on MLR

Case no.	Lymphocytes preincubated with medium alone*	Lymphocytes preincubated with indium tropolone*	Lymphocytes preincubated with indium oxine*
1	4.14	4.12	4.09
2	4.13	4.08	4.00
3	4.16	4.00	4.10
4	4.04	3.96	3.95
5	3.72	3.53	3.53
6	3.89	3.75	3.66
7	3.46	3.26	3.15
8	4.51	4.45	4.04
9	4.63	4.52	3.79
10	3.98	3.88	3.84
11	3.97	3.89	3.86
12	3.69	3.81	3.64
13	3.96	4.09	3.87
14	3.48	3.51	3.56
15	4.18	4.26	4.21
Mean ± s.d.	4.00 ± 0.32	3.94 ± 0.34	3.82 ± 0.27
Paired t-test†		$p < 0.06$	$p < 0.01$

* Values are expressed as logarithmic convertion of ^{125}I deoxyuridine uptake by lymphocytes.

† Paired t-test was calculated compared with lymphocytes preincubated with medium alone.

It is difficult to compare lymphocyte data with data relating to neutrophils labeled with indium tropolone or indium oxine (*10*,*19*) on account of the different functional and morphological characteristics of the cells in question. Gunter et al. (*19*) have reported that the indium-oxine complex is preferable to the use of indium tropolone when labeling human neutrophils. In their study, cells which were incubated with tropolone for 15 min (as compared to 7 min in our study). Burke et al. (*10*), on the other hand, have reported a higher toxicity when oxine is used.

In conclusion, no significant difference was noted between the capacity of the two chelates to impair cellular function. In the case of lymphocytes, indium oxine would seem to be slightly more toxic, at least under present experimental conditions. In our opinion indium tropolone is more suitable for lymphocyte labeling mainly because the incubation is carried out in plasma and less time is required for labeling in comparison to oxine.

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