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Adoptive Transfer of Lymphokine-activated Killer Cells Loaded with 4′-Deoxy-4′-iododoxorubicin: Therapeutic Effect in Mice Bearing Lung Metastases[1]

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ABSTRACT

We studied the potential use of lymphokine-activated killer (LAK) cells loaded with 4′-deoxy-4′-iododoxorubicin (IDX) in adoptive immunotherapy experiments. Because LAK cells preferentially locate in the lung, we evaluated the therapeutic effect of IDX-loaded LAK cells in mice bearing lung metastases induced by B16F1 tumor cell injection. *In vitro* studies showed that LAK cells rapidly incorporated IDX, with maximum uptake at 15 min, followed by a plateau; drug efflux was initially rapid and then continued at a much slower rate. Evaluation of LAK cell cytotoxic activity against relevant target cells showed a 30% decrease after IDX treatment that progressed with time over the next 6 h. P388 tumor cell growth was inhibited by coculture with IDX-loaded LAK cells, thus demonstrating that the released IDX maintained its pharmacological activity. Finally, high performance liquid chromatography analysis of tissue IDX concentration revealed a considerably higher and long-lasting concentration in the lungs of mice receiving IDX-loaded LAK cells, compared to mice given injections of a comparable amount of free drug. Moreover, adoptive transfer of IDX-loaded LAK cells into tumor-bearing mice caused a significant reduction in the number of lung metastases *versus* control mice given injections of even higher doses of free drug.

INTRODUCTION

A major drawback in the chemotherapeutic approach to cancer is that the drugs used have dose-limiting side effects. To overcome this obstacle and increase the therapeutic index, many attempts have been made to deliver the antineoplastic drugs directly to the tumor site. Liposomes (1) and RBC (2) have been used as carriers, because they allow a slow drug release and tend to concentrate in organs, such as the liver, spleen, and bone marrow, that are rich in reticuloendothelial cells and fenestrated capillaries. Drugs entrapped in these carriers, therefore, are particularly effective in treating tumors arising in or infiltrating these organs. However, by using the new generation of liposomes, referred to as Stealth (3), a long circulation time, a reduced uptake by the reticuloendothelial system, and a relative selectivity in tumor localization was achieved (4).

We showed that after preincubation with ricin, tumor-specific CTL[4] and LAK cells could internalize the toxin and release it into the culture medium without any alteration in its activity. This finding suggested that lymphocytes might also be used to deliver toxic substances to the tumor site (5, 6). Indeed, following the adoptive transfer of tumor-specific CTL loaded with ricin into mice bearing tumors induced by virus injection in the footpad, we observed a temporary inhibition of tumor cell growth. The efficacy of this treatment, however, was in part impaired by the distribution pattern of the transferred cells that, following i.v. injection, were trapped in the host lungs, thus considerably reducing the number of cells that could reach the tumor mass (5).

Nonetheless, we utilized the preferential pulmonary localization of *in vitro*-activated lymphocytes to treat animals given injections of tumor cell lines that metastatize to the lung, and we observed a significant decrease in the number of metastases in mice receiving an adoptive transfer of ricin-treated LAK cells (6). However, the fine line separating the therapeutic from the toxic effects of ricin precluded the use of toxins in this approach and led us to search for cytotoxic agents for which the high uptake by LAK cells was combined with a lower intrinsic toxicity.

In this study we show that IDX, a highly lipophilic anthracycline (7), can be internalized by LAK cells and then released in a still active form. The adoptive transfer of IDX-loaded LAK cells into mice bearing lung metastases brought about a drastic decrease in the number of metastatic nodules. This chemoimmunotherapeutic effect was related to the higher and long-lasting IDX concentration achieved in the lungs by the transferred cells, compared to levels reached with an equivalent dose of free drug.

MATERIALS AND METHODS

Mice. Inbred C57BL/6 and DBA/2 mice 6 to 8 weeks old were purchased from Nossan (Correzzana, Milan, Italy). The weight of the C57BL/6 mice used in the adoptive immunotherapy experiments ranged from 17 to 19 g.

Tumor Cell Lines. P815 (H-2^d), a chemically induced mastocytoma, was maintained by weekly passages of the ascites form in syngeneic DBA/2 recipient mice. The P388/S tumor cell line (H-2^d) and the anthracycline-resistant subclone P388/R were kindly supplied by Farmitalia Carlo Erba (Milan, Italy) and maintained by *in vitro* passages in complete medium. Complete medium consisted of Dulbecco's modified Eagle's medium (GIBCO BRL, Gaithersburg, MD) supplemented with 2 mM L-glutamine, HEPES 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 50 μM) 2-mercaptoethanol, antibiotics, and 10% heat-inactivated FBS (Irvine Scientific, Santa Ana, CA). The B16F1 tumor cell line (H-2^b) (8) was maintained in monolayer culture in complete medium.

LAK Cell Generation. LAK cells were generated by culturing 3×10^6 C57BL/6 spleen cells/ml with 200 units/ml recombinant IL-2 kindly supplied by EuroCetus (Milan, Italy) for 5 days in complete medium at 37°C in a 5% CO_2 water-saturated atmosphere.

Evaluation of Drug Uptake and Release by LAK Cells. IDX was a gift of Farmitalia Carlo Erba. LAK cells (10^6/ml) were incubated at 37°C with 10 μM IDX in medium containing 3% FBS for different time intervals. To stop IDX influx, incubation was terminated by rapid chilling to 4°C, and cells were centrifuged through a layer of FBS to remove extracellular drug.

To evaluate drug release, cells were incubated for 30 min as reported above, washed, resuspended in drug-free medium, and incubated at 37°C for varying times. To arrest efflux, aliquots were removed, chilled, and centrifuged through FBS. Drug content in the cell pellet was determined by HPLC analysis as described previously (9). Briefly, 1.0 ml extraction buffer (0.6 N HCl:ethanol, 50:50) was added to the cell pellet; the mixture was shaken for 10 min at 4°C

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[4] The abbreviations used are: CTL, cytotoxic T-lymphocytes; IDX, 4′-deoxy-4′-iododoxorubicin; FBS, fetal bovine serum; HPLC, high-performance liquid chromatography; LAK, lymphokine activated killer; IL-2, interleukin 2; LD_{50}, 50% lethal dose; E:T, effector:target cell ratio.

followed by centrifugation at 14,000 × *g*, and the supernatant was injected into the column. We used a reverse phase HPLC system with a C_{18}-μBondapak column and a C_{18}-GuardPack precolumn; the LS4 fluorimeter (Perkin Elmer, Norwalk, CT) was set at 479 nm excitation and 593 nm emission wavelengths. Peaks were quantified by an LC 100 integrator SP4270 (Spectra-Physics, Fremont, CA). The mobile phase was 0.05 M 69% KH_2PO_4-31% CH_3CN, pH 3.0; flux, 1.5 ml/min.

For the adoptive immunotherapy experiments, 3.5×10^6 LAK cells/ml were incubated for 30 min at 37°C with 10 μM IDX in medium containing 3% FCS. Before inoculation, the LAK cells were held at room temperature for 10 min in order to eliminate loosely bound IDX, washed, and resuspended in cold medium at a concentration of 12.5×10^7 cells/ml; an aliquot of cells was evaluated for IDX content by HPLC.

Cytolytic Assay. LAK cell cytotoxic activity was evaluated by incubating serial dilutions of effectors with 2×10^3 ^{51}Cr-labeled ($Na_2{}^{51}CrO_4$; NEN, Boston, MA) P815 target cells in triplicate wells of round-bottomed microtiter plates (Sterilin Bibby, Hounslow, United Kingdom) in a final volume of 0.2 ml, as described previously (10). After 4 h, the plates were centrifuged, and 0.1 ml supernatant was removed for counting. The percentage of specific ^{51}Cr release was calculated as

$$\frac{\text{Experimental release} - \text{spontaneous release}}{\text{Maximum release} - \text{spontaneous release}} \times 100$$

Tumor Cell Proliferation Assay. LAK cells were incubated at 37°C with 10 μM IDX for 30 min, then chilled at 4°C, centrifuged through a layer of FBS, and resuspended in drug-free medium. IDX-treated LAK cells were held at room temperature for 10 min, in order to eliminate loosely bound IDX, then washed and resuspended in complete medium. P388/S or P388/R tumor cells (5×10^3) were incubated with serial dilutions of IDX-treated or untreated LAK cells in a final volume of 0.2 ml in triplicate wells of flat-bottomed microtiter plates (Sterilin). After 40 h, the cultures were pulsed with 1 μCi [^{3}H]thymidine/well (NEN) and collected 8 h later on filter paper using a cell harvester (Skatron AS, Trarnby, Norway). Radioactivity was determined in a β-scintillation counter (Beckman Instruments, Inc., Irvine, CA).

Evaluation of IDX Content in Tissues. Mice were sacrificed by excess ethyl ether anesthesia; lungs and other organs were removed and washed repeatedly in 0.9% NaCl solution. Drug extraction from organs was performed as follows. After addition of 1.0 ml of distilled water, tissues were homogenized using an Ultra Turrax apparatus (Janke U. Kunkel, Staufen, Germany). Ten μl of doxorubicin (10 μg/ml) as internal standard, 200 μl of 4 M NaCl, and 100 μl of 0.05 M H_3PO_4 were added to 200 μl of homogenate and mixed vigorously for 5 min at 4°C. Extraction mixture (CH_3CN:methanol:H_2O, 50:30:10) (490 μl) was then added, and the samples were shaken for 10 min at 4°C. Tubes were spun at 14,000 × *g* for 10 min, and the supernatants were injected into HPLC as above.

Adoptive Immunotherapy. B16F1 cells were harvested with medium containing 0.25% trypsin and 1 mM EDTA, washed, and resuspended in medium; 0.2 ml of this cell suspension, containing 2×10^5 cells, was injected i.v. into C57BL/6 mice. Two days later, the mice were inoculated i.v. with LAK cells that had been pretreated with IDX for 30 min. Control mice were given injections of untreated LAK cells or different amounts of free IDX. Mice were sacrificed from 16 to 22 days after tumor cell inoculation; the lungs were removed, rinsed in water, and fixed in Bouin's solution. B16F1 metastases formed black nodules on the lung surface and were counted after fixation at the microscope under low power (×16) in a blind fashion.

RESULTS

IDX Uptake and Release by LAK Cells. The kinetics of IDX uptake by LAK cells was evaluated by means of HPLC analysis. As shown in Fig. 1*A*, LAK cells rapidly incorporated IDX; a high uptake was obtained at 15 min, after which values reached a plateau, and only a small increase in uptake was observed by prolonging the incubation up to 45 min. On the basis of these findings, we used a 30-min incubation time to load LAK cells in the subsequent studies. Under these conditions, the variation in IDX incorporation in all the experiments performed (8 experiments) was 2345 ± 436 (SD) ng/10^6 LAK cells. The main reason of variation in the uptake was the heterogeneity

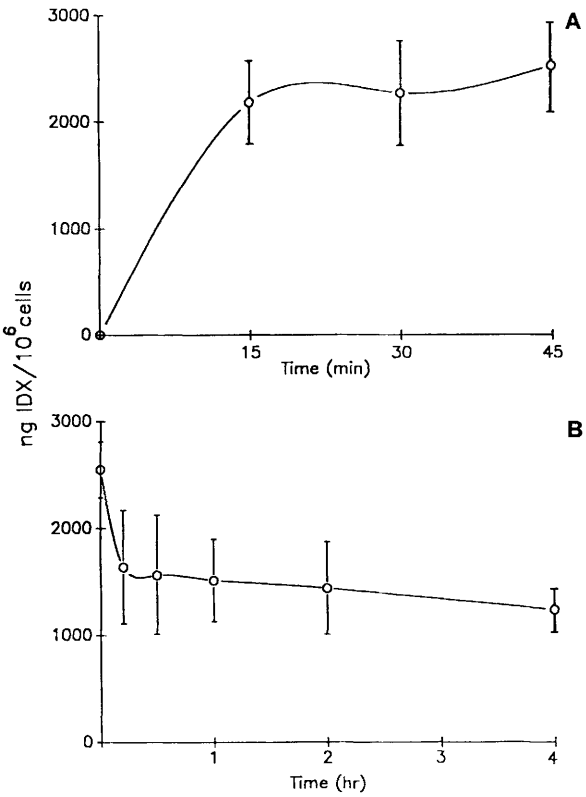

Fig. 1. Time course of uptake (*A*) and release (*B*) of 10 μM IDX by LAK cells evaluated by HPLC analysis. In *A*, LAK cells were treated with the drug for the indicated times, and incubations were terminated by rapid chilling at 4°C; extracellular drug was removed by centrifuging the cells through a layer of FBS. Data represent the average values ± SD (*bars*) of three separate experiments. In *B*, LAK cells were treated with the drug for 30 min, washed, and then resuspended in drug-free medium; IDX efflux was evaluated at the indicated time. Data represent the average values ± SD of three separate experiments.

in the size of the LAK cell populations used, which was related to the number of activated cells in the different cultures.

Fig. 1*B* shows the kinetics of drug efflux from IDX-loaded LAK cells, evaluated in 3 different experiments. Drug release was initially rapid and was followed by a much slower efflux rate. Indeed, by 10 min approximately 35% of the total drug incorporated by the LAK cells was released, while after 4 h only 50% was lost. Thus, part of the IDX remained tightly bound within the cells. A similar two-phase kinetics of drug efflux was described by others for IDX-sensitive tumor cell lines (11).

***In Vitro* Tumor Cell Lysis and Growth Inhibition by IDX-treated LAK Cells.** To study whether IDX pretreatment affected LAK cell lytic activity, we evaluated LAK cell ability to kill P815 target cells at different time intervals after IDX treatment by means of a ^{51}Cr release assay. Compared to untreated LAK cells, IDX-treated cells showed a 30% reduction in cytotoxic activity at the end of the incubation period and a gradual loss of activity in the following 6 h (Fig. 2). We then investigated whether the IDX released by the LAK cells conserved its capacity to inhibit tumor cell growth. Therefore, increasing numbers of IDX-treated LAK cells were added to cultures of the anthracycline-susceptible tumor cell line P388/S, or the resistant subclone P388/R and tumor cell growth was evaluated after 48 h. The P388/S cell line showed a dose-dependent growth reduction while, as expected, the P388/R subclone was less susceptible to inhibition (Fig. 3*A*). Indeed, the number of IDX-loaded cells required to obtain a 50% reduction in P388/R cell growth was 6-fold higher than that which produced a comparable effect on the P388/S cells. The addition of untreated LAK cells did not affect tumor cell growth, most likely because even in cultures to which the highest LAK cell number

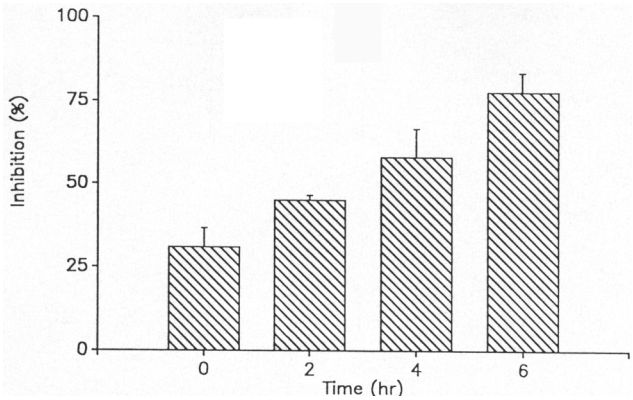

Fig. 2. Inhibition of IDX-treated LAK cell lytic activity. The evaluation was carried out against P815 target cells at the indicated times after IDX treatment. The percentage of cytotoxicity was determined in a 4-h ^{51}Cr release assay at an E:T ratio of 100:1; inhibition is expressed as a percentage compared to the untreated LAK cells. *Bars*, SD.

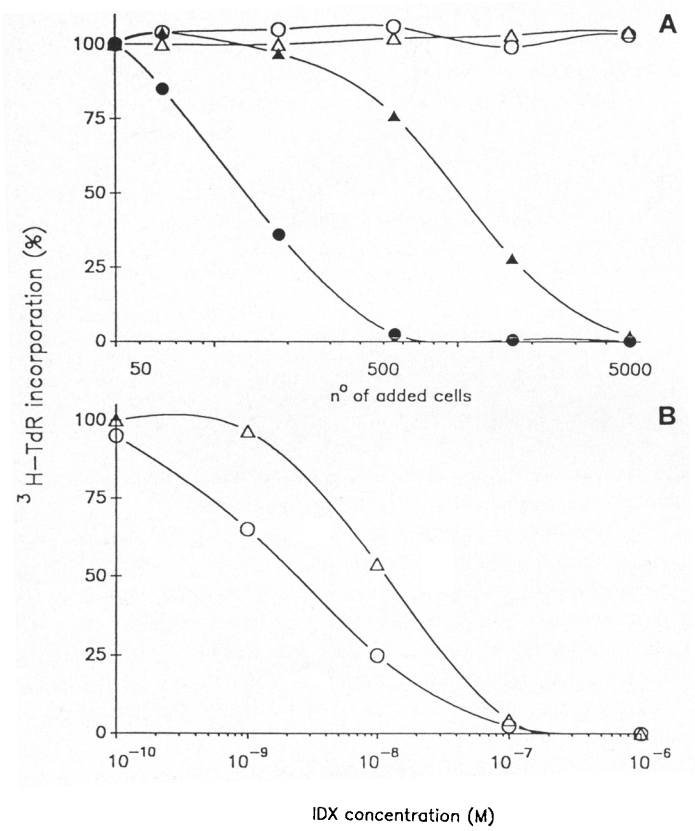

Fig. 3. [^{3}H]Thymidine incorporation of P388/S and P388/R tumor cells. In *A*, P388/S (○, ●) and and P388/R (△, ▲) cell growth was evaluated in the presence of different numbers of untreated (○, △) or IDX-treated (●, ▲) LAK cells. In *B*, P388/S (○) and P388/R (△) tumor cells were cultured in the presence of the indicated concentration of free IDX. The results are expressed as percentage growth, compared to control cultures containing only tumor cells.

was added, the E:T cell ratio was too low (1:1) to induce a relevant tumor cell killing. Only by increasing the effector cell number we observed a gradual inhibition of tumor cell growth, as 50% reduction was achieved at an E:T ratio of 15:1 (data not shown).

Parallel experiments, in which free IDX was added to cultures, confirmed the different sensitivity of the two cell lines to anthracyclines (Fig. 3*B*). Indeed, to achieve 50% growth inhibition, the resistant cell line required a 5-fold higher concentration of IDX than that needed by the susceptible one (12 nM *versus* 2.4 nM, respectively). In different experiments the estimated concentration of drug released by LAK cells that determined 50% P388/S tumor cell growth inhibition ranged from 3 to 3.7 nM and was similar to the concentration of free drug (ranging from 1.6 to 2.4 nM) that produced a comparable effect. These findings suggest that most of the drug released by LAK cells maintained its pharmacological activity.

Selective Accumulation of IDX in the Lungs of Mice following Adoptive Transfer of IDX-loaded LAK Cells. Adoptive transfer studies of *in vitro*-activated lymphocytes showed that most of the transferred cells localized in the host's lungs shortly after i.v. injection and reached the liver and spleen only after several h (12–15).

In the light of this distribution pattern, we examined the possibility of obtaining selective drug accumulation in the lungs of recipient animals through the transfer of IDX-loaded LAK cells. To this end, we compared IDX contents in the lungs of mice that were given i.v. injections of IDX-treated LAK cells or different doses of free drug (from 40 to 160 μg). To evaluate IDX distribution, the animals were sacrificed at various intervals after injection, and drug concentration in different organs was determined by HPLC analysis. In the experiment reported in Fig. 4, which is representative of 2 others that gave

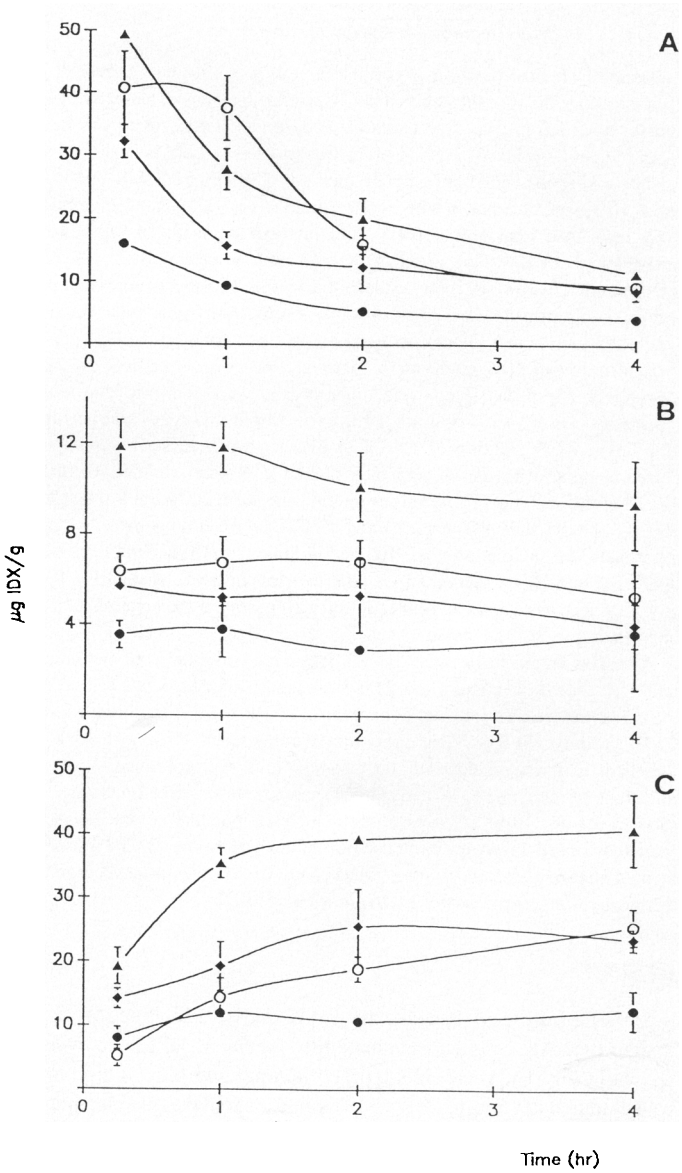

Fig. 4. IDX levels in the organs of mice treated with IDX-loaded LAK cells or free IDX. Drug levels in lung (*A*), liver (*B*), and spleen (*C*) of C57BL/6 mice at different times after injection of 30×10^6 IDX-treated LAK cells (○) and 40 μg (●), 80 μg (◆), and 160 μg (▲) of free IDX. Data reported for the liver represent the sum of IDX levels and its two major metabolites. *Points*, mean ± SD (*bars*) of three mice/experimental group. Where no *error bars* are seen, the SD is smaller than the size of the symbols.

Table 1 *Antitumor effect of IDX-treated LAK cell adoptive transfer*

Statistical significance: in experiment 1 IDX 70 μg *versus* LAK-IDX, $P = 0.0017$; in experiment 2 IDX 40 μg *versus* LAK-IDX, $P = 0.0075$; in experiment 3 IDX 40 μg *versus* LAK-IDX, $P = 0.001$ by Mann-Whitney-Wilcoxon test.

Treatment	No. of lung metastases[a]					
	Expt. 1	Mean	Expt. 2	Mean	Expt. 3	Mean
None	27, 30, 33, 46, 54, 56, 62, 66, 66	49	15, 20, 25, 35, 84	36	14, 19, 22, 24, 30, 40, 44, 58, 65	35
IDX, 40 μg	ND		12, 13, 19, 20, 23	17	9, 12, 13, 14, 15, 23, 24, 33, 41	20
IDX, 70 μg	6, 7, 9, 10, 13, 14, 27, 27, 29	16	ND		6, 10, 17, 17, 19, 22, 31, 44	21
LAK-IDX[b]	0, 0, 1, 1, 2, 3	1	1, 1, 1, 2, 3, 4	2	0, 0, 0, 2, 2, 2, 8	2
LAK[c]	28, 35, 45, 56, 58, 61	47	ND		ND	

[a] Mice receiving i.v. 2×10^5 B16F1 tumor cells were sacrificed on day 17 in experiment 1, on day 22 in experiment 2, and on day 19 in experiment 3, and lung metastases were counted.

[b] Two days after tumor cell injection, recipient mice were inoculated i.v. with 50×10^6 LAK cells pretreated with 10 μM IDX for 30 min. LAK cells contained 72, 32, and 36 μg of IDX in experiments 1, 2, and 3, respectively.

c Two days after tumor cell injection, recipient mice were inoculated i.v. with 50×10^6 LAK cells.

comparable results, the 30×10^6 IDX-treated LAK cells that were transferred contained 49.8 μg IDX. 15 min after injection, the IDX level in the lungs of mice given injections of these cells was 2.5-fold higher than that of mice given injections of a comparable dose (40 μg) of free drug, and within the range of values determined by the injection of 80 or 160 μg free IDX (Fig. 4*A*). Interestingly, after 1 h, these differences in IDX level were even more striking, as the concentration of the drug in the lungs of mice receiving IDX-loaded LAK cells remained high but decreased sharply in mice treated with the free drug. At this time, the amount of IDX delivered by LAK cells was 1.4-fold higher than that reached in mice given injections of 160 μg free drug which corresponded to the LD_{50}. In the following three hours, the IDX concentration also decreased in mice receiving IDX-loaded cells and settled within the range determined by the injection of 80 and 160 μg free drug.

Pharmacokinetics studies in the liver revealed rather constant drug levels over the period considered; in mice given injections of IDX-treated LAK cells, the concentration reached was slightly higher than that reached with 80 μg IDX (Fig. 4*B*). The evaluation of IDX content in the spleen disclosed a very low drug level 15 min after treatment in mice given injections of loaded cells. However, the concentration of the drug progressively increased and by 4 h reached the same level observed in mice given injections of 80 μg free drug (Fig. 4*C*). The presence of the major IDX metabolites, 13-dihydro-IDX and 7-deoxyadriamycinol aglycone (9, 16), was also determined in the same experiment. These metabolites were undetectable in the lung. 13-Dihydro-IDX was detected in the spleen only 4 h after injection, where it represented only 1–2% of the concentration of free IDX; 7-deoxyadriamycinol aglycone was undetectable. Both metabolites were present in the liver and accounted for 15–30% of the total drug amount, but their kinetics was similar to that observed with IDX.

Inhibition of Lung Metastases Growth by Adoptive Transfer of IDX-loaded LAK Cells. We evaluated the therapeutic effect of IDX-loaded LAK cells by means of adoptive cell transfer in mice given injections of B16F1 melanoma, a cell line which is metastatic in the lung (8) and susceptible to anthracyclines (17). Two days after tumor cell injection, the mice were inoculated with 50×10^6 IDX-loaded LAK cells. Control mice received either an equal number of untreated LAK cells or an amount of free IDX corresponding to that carried by LAK cells.

The results of 3 different experiments are reported in Table 1. In every case, the number of lung metastases was significantly reduced in mice receiving IDX-loaded LAK cells, compared with mice receiving an equivalent amount of free drug. Moreover, 2 of 6 and 3 of 7 mice in experiments 1 and 3, respectively, apparently were free of metastases. Untreated LAK cells, without contemporary administration of IL-2, had no protective effect as observed in preliminary experiments in which a different anthracycline (doxorubicin) was used (18).

Finally, in mice receiving different free IDX doses (experiment 3), no reduction in the number of metastases was observed with increasing drug amounts; indeed the reduction in the number of metastatic nodules induced by 70 μg IDX was almost equal to that achieved with 40 μg (21 *versus* 20). Moreover, in the group of mice treated with 140 μg free IDX, which was close to the LD_{50}, the number of metastases in the survivors was only slightly reduced (17 ± 12) (data not shown).

DISCUSSION

We previously demonstrated that cytotoxic cells (CTL or LAK cells) could incorporate ricin in their cytoplasm and then release it into the culture medium in its active form (5, 6). We also showed that when ricin-loaded cytotoxic cells were adoptively transferred into tumor-bearing mice, they were able to either confer transient protection or regress the tumor (5, 6). These encouraging results were in part vitiated by the need to use the entire ricin molecule, in order to obtain its uptake by carrier cells; thus the nonspecific binding due to the B-chain reduced the narrow margin existing between the therapeutic activity and high toxicity of this molecule (19).

The present findings constitute an improvement in this approach, inasmuch as we succeeded in loading cytotoxic cells with a chemotherapeutic agent which, compared to ricin, exerts a well defined anticancer activity and has a lower toxicity. IDX, a new anthracycline derivative obtained from a chemical modification of doxorubicin, was preferred to doxorubicin because its high lipophilia allows a more rapid and higher incorporation into cells (7). Indeed, compared to doxorubicin, IDX uptake by LAK cells was 5 times higher, while efflux was similar.[5]

Although, LAK cell viability following IDX incorporation was not altered for up to 8 h after treatment (evaluated by the dye exclusion assay, data not shown), cytotoxic activity was progressively impaired, and by 6 h it was almost abrogated. The *in vitro* findings also indicated that the pharmacological activity of the IDX released by the LAK cells was unaltered. In fact, to achieve 50% inhibition of P388/S tumor cell growth, comparable amounts of free IDX or IDX incorporated by LAK cells were required. Tumor cell growth inhibition was due exclusively to the pharmacological activity of the released IDX, inasmuch as LAK cell lytic activity by itself was irrelevant; indeed, at the E:T cell ratio used, untreated LAK cells did not induce inhibition of tumor cell growth; moreover, P388/R and P388/S cell lines, although

[5] S. Mandruzzato, unpublished results.

equally susceptible to LAK cell lytic activity (data not shown), showed a different susceptibility to inhibition in the growth pattern when cocultured in the presence of IDX-loaded LAK cells.

In previous studies on the distribution of transferred cytotoxic cells, we observed that most of the i.v.-injected cells are rapidly trapped in the lungs of recipient mice and thereafter migrate to the liver and spleen (5, 6). Accordingly, evaluation of the tissue IDX concentration revealed that the IDX level in the lungs of mice receiving IDX-loaded cells was considerably higher than that observed in mice given injections of a comparable amount of free drug; moreover, these high levels persisted for a longer time, suggesting that IDX is slowly released in this site by the transferred cells. One h after cell injection, we observed higher tissue levels than those reached with 160 μg of free IDX, which is the LD_{50}. The IDX concentration in the livers of mice treated with loaded LAK cells was higher than that of mice given injections of a corresponding amount of free IDX, but IDX pharmacokinetics in the two groups of mice were similar. In the spleen, where LAK cells were shown to localize much later (12), the IDX level was very low 1 h after injection but increased progressively during the next 4 h.

The persistingly high drug levels reached in the lung when IDX is delivered by LAK cells not only constitute the advantage of this approach but also clearly explain the significantly greater reduction in the number of lung metastases, compared to treatment with the same amount of free IDX. Indeed, even the injection of an IDX dose 3 to 4 times higher than that carried by transferred LAK cells did not induce a comparable reduction in metastatic nodules. In fact, mice that survived the administration of the toxic dose of 140 μg free IDX still had a higher number of lung metastases than mice receiving one-fourth of this dose via LAK cell transfer (data not shown). Preliminary experiments in which mice bearing advanced metastases were given i.v. injections of 3 doses of IDX-loaded LAK cells not only disclosed a significant increase in survival time, compared to mice receiving an equal amount of free drug, but also a cure rate of 25%.[6]

In conclusion, our findings confirm that lymphocytes constitute suitable carriers for the delivery of toxic substances or drugs to the tumor site. The disadvantage of this method, like that of others based on drug encapsulation in particulate vectors, consists in the fact that vector localization is governed mainly by their tissue distribution pattern. To overcome this limit, experiments are in progress in which, prior to transfer, drug-loaded LAK cells are pretreated with bispecific monoclonal antibodies directed against a LAK cell receptor and a tumor-specific antigen (20, 21). By means of this procedure, it might be possible to associate the advantages of a preferential tumor localization with the capacity of the transferred LAK cells to release molecules endowed with a high anticancer activity; it is conceivable that this procedure might even affect bystander tumor cells, which do not come directly into contact with the effector cells targeted by the bispecific monoclonal antibody.

[6] S. Mandruzzato *et al.*, manuscript in preparation.

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