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Etoposide-induced DNA cleavage in human leukemia cells*

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Summary. The nuclear enzyme, topoisomerase II, is the major site of action for cancer chemotherapy agents such as etoposide, teniposide, and a variety of intercalating agents. These compounds cause the enzyme to cleave DNA, forming a DNA-protein complex that may be a key step leading to cell death. It is apparently unique as a chemotherapy target, since drug potency diminishes with decreasing enzyme activity. It was thus of interest to examine the topoisomerase content and drug-induced DNA cleavage in freshly obtained human leukemia cells and to compare the obtained data with the results of similar studies performed in well-characterized human leukemia cell lines. The human T-lymphoblast line, CCRF-CEM, was more than 100-fold more sensitive to the DNA-cleavage effect of etoposide than the cells of the 13 leukemic patients examined. One of the leukemia lines (HL-60) and a lymphoblastoid line (RPMI-7666) were somewhat less sensitive than cells of the CCRF-CEM cells, but were still 10-fold more sensitive than the patients studied. The relative insensitivity of the freshly obtained cells could not be accounted for by differences with respect to drug uptake but were associated with markedly reduced topoisomerase-II content as assayed by immunoblotting using a mouse polyclonal serum against topoisomerase II. Heterogeneity was observed in the sensitivities of patients' cells with respect to both drug-induced DNA cleavage and enzyme content. The observed differences between cultured cell lines and patients' cells may have been related to their proliferative status. Etoposide potency in normal resting lymphocytes resembles that observed in circulating leukemia cells. However, following mitogenesis with phytohemagglutinin and interleukin-2, proliferating lymphocytes become as sensitive to etoposide as cultured cell lines with regard to DNA cleavage. This effect was accompanied by an increase in topoisomerase-II content. Our data thus support the hypothesis that topoisomerase-II content may be an important determinant of cell sensitivity to certain classes of chemotherapy agents. Efforts to stimulate topoisomerase-II content may improve the therapeutic efficacy of these drugs.

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Introduction

The nuclear enzyme DNA topoisomerase II, has recently been recognized as being a critical target for the action of a number of clinically useful anticancer agents [15, 25]. This enzyme is an ATP-dependent homdimer which reversibly catalyzes the co-ordinated breakage and resealing of double-stranded DNA. During this reaction, an intermediate is formed, in which the enzyme is covalently linked to the DNA, and this intermediate allows the passage of another double-stranded segment of DNA through the complex. The enzyme's action allows the segregation of interlocked DNA molecules and appears to be critical for the successful conclusion of DNA replication [6, 8]. In the presence of certain anticancer agents, e. g., intercalating agents and epipodophyllotoxins, topoisomerase II remains bound to the DNA [12, 16]. Denaturation of this complex reveals protein-associated DNA breaks. Available evidence strongly suggests that the perpetuation of this DNA-protein complex sets into motion a series of events which lead to cell death. One of the most novel features of this enzyme as a chemotherapy target is that its presence is required for the expression of drug cytotoxicity. Indeed, the enzyme is actually a fundamental part of the lethal process and, in contrast to other enzymes which serve as chemotherapy targets, drug sensitivity increases as a function of this enzyme's activity [7, 13, 22].

There is little available information concerning the intracellular topoisomerase content of human malignancies and its relationship with drug effects. Priel et al. [14] have reported that topoisomerase II activity is undetectable in resting normal lymphocytes but is fully expressed in a variety of human leukemic and lymphoblastoid cell lines. They concluded that the leukemia cells contain an excessive level of topoisomerase II, although the enzyme content was not actually measured. A variety of studies have suggested that topoisomerase II activity is dependent on the proliferative status of a cell, however, and this may have an important bearing on the findings of Priel et al. work [11, 22, 23].

In order to assess the sensitivity of human leukemia cells to the epipodophyllotoxin, etoposide, and to correlate this sensitivity with the cellular content of the target enzyme, we examined freshly obtained human leukemia cells from patients with a variety of diagnoses and compared them to cultured human leukemia cell lines with respect to drug-induced DNA cleavage, drug uptake, and

enzyme content. In addition, we found further evidence for the importance of proliferation in determining drug sensitivity and topoisomerase content.

Materials and methods

Patients. A total of 13 patients with various forms of leukemia were sampled, i. e., 6 with chronic lymphocytic leukemia , 3 with acute lymphocytic leukemia (ALL), 3 with acute myelogenous leukemia (AML), 1 with hairy-cell leukemia, and 1 with chronic myelogenous leukemia (CML) in blast crisis. No patient had received chemotherapy less than 3 weeks before the study. Patients samples were assayed for DNA cleavage, intracellular drug content, and topoisomerase-II content; however, not all cases provided sufficient cells for all types of analysis.

Cell preparation. Human peripheral whole blood (12–40 ml) was obtained in a sterile heparized tube, placed on ice, and diluted with cold, sterile phosphate-buffered saline (PBS) to a final volume of 90 ml. Monocytes were depleted by adhesion to plastic for 1 h at 37 °C. Peripheral mononuclear cells were then isolated by centrifugation through LSM (Lymphocyte Separation Medium; Litton Bionetics, Kensington, Md). The buffer layer was captured, washed with PBS, and resuspended in RPMI-1640 plus 10% fetal calf serum (FCS) at a cell density of approximately 2×10^6 cells/ml. Cells were counted using a hemocytometer, and their viability was assayed by trypan-blue dye exclusion. Cells were used for the following experiments within 24 h of being obtained; no experiments were performed on cell preparations with less than 90% viability.

The human cell lines and the media used for their support included HL-60 cells, an acute promyelocytic line maintained in RPMI 1640 containing 10% FCS, CCRF-CEM, a T-cell lymphoblastic leukemia line maintained in α-MEM with 10% FCS, and RPMI-7666, a B-lymphoblastoid line maintained in RPMI 1640 plus 20% FCS.

Drug treatment. Cells were incubated with various concentrations (0–50 μ*M*) of etoposide (Bristol-Myers, Syracuse, NY) at a cell density of 1.5×10^6 cells/ml for 1 h at 37 °C, and were then placed in 20 ml cold PBS on ice and used immediately for fluorometric alkaline elution.

Cell irradiation. Cells were irradiated in a Mark-I Model-35 Irradiator (J. L. Shephard and Associates, Glendale, Calif) using a standard cesium source; the cells were kept on ice to prevent repair of DNA strand breaks. Doses of 0–1,200 rad were delivered by varying the time and intensity of exposure to the cesium source.

Fluorimetric alkaline elution. Our method was a variation of that described by Stout and Becker [21]. In brief, $1.5–3.0 \times 10^6$ cells were poured over an empty column onto a 25-mm polycarbonate filter (pore size 2 μ*M*; Nuclepore, Pleasanton, Calif), washed with PBS, and subsequently lysed for 15 min with 2 ml 0.2% Sarkosyl (ICN Pharmaceuticals, Plainview, NY), 0.4 *M* Na-ethylenediaminetetraacetate (EDTA), 2 *M* NaCl, and 0.5 mg/ml proteinase K (Sigma, St. Louis, Mo). The cells were then washed twice with 5 ml 0.02 *M* Na-EDTA, pH 10.0, and the DNA was eluted with 24 ml tetrapropylammonium hydroxide (RSA, Ardsdale, NY), pH 12.1, containing 0.02 *M* Na_2-EDTA. Five 3-ml fractions were collected at a rate of 0.03 ml/min. Unused eluting solution was then decanted from the columns, the lines were emptied into another fraction, and the filters were placed into tetrapropylammonium solution and heated for 30 min at 55 °C to remove remaining DNA. Three-milliliter samples were then neutralized with 1.0 *M* KH_2PO_4 to a pH of 6.8–7.0, and 0.8 ml 2.25 μ*M* Hoechst – 33258 dye (Calibiochem) was added. Samples for standard curve construction were prepared in the same solutions using calf thymus DNA (0–1.0 μg/ml), and all samples were read on a Perkin-Elmer LS2 Fluorimeter (Perkin-Elmer, Bucks., UK) using an excitation wavelength of 350 nm and an emission wavelength of 461 nm.

^{3}H-Etoposide cellular uptake. This has been described in detail elsewhere [26]; in brief, 3×10^8 cells in 2 ml media were incubated for 1 h at 37 °C with 10 or 50 μ*M* ^{3}H-etoposide (Moravek Biochemicals, Brea, Calif). The cells were then spun down, washed, dried, weighed, and placed into scintillation fluid for counting.

Nuclear extraction of topoisomerase II. All procedures were performed at 0 °C, and fresh phenylmethylsulfonyl fluoride (PMSF; Sigma) was added to all buffers at a final concentration of 1 m*M*. Between 1×10^9 and 1×10^9 cells were washed in 0.15 *M* NaCl and 10 m*M* KH_2PO_4 and then centrifuged at 4,000 rpm for 10 min. The cells were then suspended in buffer B [5 m*M* KH_2PO_4, 2 m*M* $MgCl_2$ – 6 H_2O, 4 m*M* dithiothreitol (DTT), and 0.1 m*M* EDTA] for 30 min and subsequently homogenized by 12 strokes in a Dounce homogenizer. After centrifugation for 10 min at 4,000 rpm, the nuclei were resuspended in 13 ml buffer C (buffer B plus 0.25 *M* sucrose), layered over 4 ml buffer D (buffer B plus 0.6 *M* sucrose), and centrifuged for 10 min at 4,000 rpm. The nuclei were resuspended in 0.5–2 ml buffer E (5 *M* KH_2PO_4, 4 m*M* DTT, and 1 mM EDTA), kept for 15 min at 0 °C, and then added to an equal volume of buffer F (buffer E plus 0.7 *M* NaCl). After maintenance at 0 °C for another 15 min, glycerol was added (10% by volume), and the sample was spun for 30 min at 13,000 rpm. The supernatant containing topoisomerase II was decanted and stored at −20 °C.

Whole cell protein determination. Whole cell preparations consisted of $1.0–4.0 \times 10^7$ cells that were washed with PBS, resuspended in 1 ml 2× sodium-dodecyl-sulfate (SDS) sample buffer (1× being 2.5% SDS, 2.5% glycerol, and 0.25 *M* Tris, pH 7.0) plus 2.5% β-mercaptoethanol and 1.0 m*M* PMSF. The cells were then vortexed and sonicated in a Braun 1510 sonicator, (Braun Melsunger) for 20 s, and the whole cell preparation was subsequently stored at −20 °C. Protein determinations were performing using the method previously described by Bensadoun and Weinstein [3]. In brief, the samples were treated with deoxycholate and then precipitated with trichloroacetic acid (TCA). The pellet was saved after centrifugation, and its protein content was determined using the Lowry method [10].

Western blotting. Nuclear extracts were mixed with 4× SDS sample buffer plus 10% glycerol, with dilution as required to achieve a final concentration of 1× SDS sample

buffer and 50 μg nuclear extract. Whole cell preparations were mixed similarly using volumes corresponding to 1×10^6 cells. Polyacrylamide gels were run at 8 W until the bromophenol-blue dye marker had left the gels. The transfer of protein from polyacrylamide to nitrocellulose was performed in transfer buffer (25 m*M* Tris, 192 m*M* glycine, and 20% v/v methanol, pH 6.5) at 4 °C for 4 h at 1 A using a 2005 Transphor unit (LKB Gaithersburg, Md). Blots were then blocked (in order to occupy the remaining protein binding sites) for 1 h at room temperature in 20 m*M* Tris-buffered saline (TBS) pH 7.5, plus 3% gelatin, and subsequently washed twice with TBS/0.05% Tween 20 (TTBS) for 5 min. The blots were incubated for 15 h in TTBS plus 1% gelatin with mouse antiserum against topoisomerase II obtained by immunizing mice with partially purified HeLa topoisomerase II. The Bio-Rad Immun-Blot assay kit (Bio-Rad Laboratories, Richmond, Calif) was used for the remainder of the procedure. After washing twice with TTBS, the blots were subjected to goat anti-mouse IgG alkaline phosphatase for 1 h, washed twice with TTBS and once with TBS, and then developed in BCIP/NBT substrat reagents (5-bromo-4-chloro-3-indolyl-phosphate sodium salt plus *p*-nitro blue tetrazolium chloride in 0.1 *M* $NaHCO_3$ and 1.0 m*M* $MgCl_2$, pH 9.8).

Interleukin-2 stimulation. Heparinized peripheral blood from normal human volunteers was overlayered onto an equal volume of Mono-Poly Resolving Medium (Flow Laboratories, McLean, Va) and centrifuged at 1.000 *g* for 30 min. The recovered mononuclear cells were washed with PBS and resuspended in primer culture medium containing RPMI 1640, 10% FCS, and 1% phytohemagglutinin (PHA; Wellcome Diagnostics, Greenville, NC) at a density of approximately 1×10^6 cells/ml. After a 48-h incubation, the cells were spun down and resuspended in medium containing 5 half-maximal units/ml interleukin-2 (human T-cell polyclone; Collaborative Research, Lexington, Mass) and 0.01 μCi/ml ^{14}C-thymidine. The cell density was maintained at $2-5 \times 10^5$ cells/ml. Incubation under these conditions supported the exponential growth of PHA-primed cells, with a doubling time of approximately 24 h. After 36 h, the cells were resuspended in label-free medium containing interleukin 2 and were incubated for an additional 12 h. They were then resuspended in fresh medium without the lymphokine. Some of these cells were reserved for Western blotting and were lysed as described below for resting lymphocytes. The remainder were treated with etoposide for 60 min at 37 °C or radiated on ice.

Drug- and radiation-induced DNA single-strand breaks were assayed using the alkaline elution technique as previously described [7]. Cells which contained ^{3}H-thymidine and had received 1,500 rad prior to elution were included on each filter as internal standards. High-frequency breaks were assayed at an elution rate of 0.16–0.20 ml/min, with a fraction interval of 7 min and a total elution time of 35 min.

Resting peripheral lymphocytes were obtained by incubating mononuclear cells in RPMI 1640 and 10% FCS for 1 h at 37 °C to allow the attachment of monocytes. The cells remaining in suspension were spun down and lysed in a solution containing 4% SDS, 0.1 *M* Tris (pH 6.8), 10% β-mercaptoethanol, and 20% glycerol. The cell lysate was then frozen at −20 °C until Western blotting was performed.

Results

Radiation dose response

Tissue-culture cells and fresh normal and leukemic human cells were exposed to a range of radiation doses as already described; DNA cleavage was subsequently measured by fluorimetric alkaline elution. A typical radiation dose response curve is presented in Fig. 1. As expected, the various cell preparations exhibited similar amounts of DNA strand breakage in response to irradiation; these data provided us with an arbitrary radiation dose standard curve from which to express drug-mediated strand breakage in terms of radiation equivalents.

Etoposide-induced DNA cleavage

Freshly obtained human leukemic cells were isolated and incubated with various doses of etoposide. Human cell lines were treated similarly, and DNA breakage was subsequently assayed by alkaline elution and expressed in radiation equivalents as already described. Figure 2 demon-

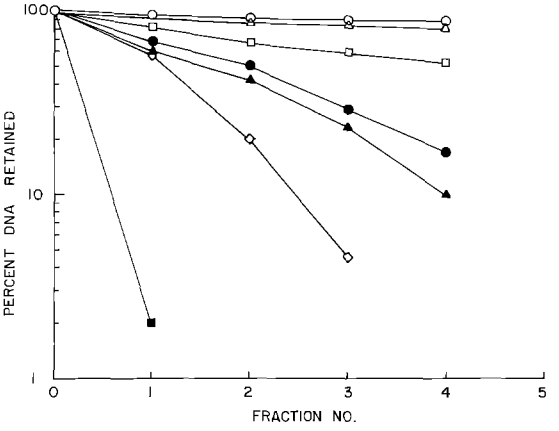

Fig. 1. Radiation dose response curve of CCRF-CEM cells. Cells were irradiated with 0 (○), 100 (△), 200 (□), 400 (●), 600 (▲), 800 (◆), or 1,000 (■) rad. The percentage of DNA remaining on the filter during alkaline elution as assayed fluorimetrically was plotted against the fraction collected

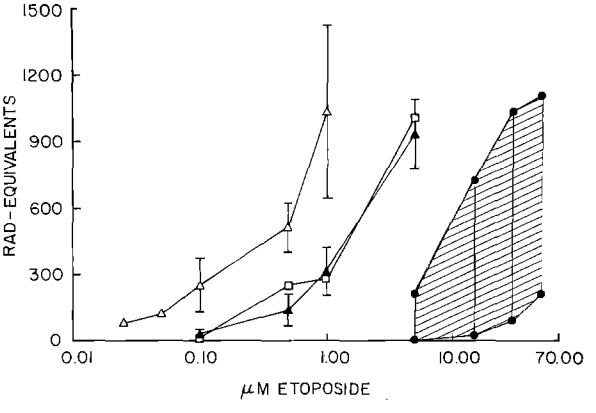

Fig. 2. Etoposide-dose response expressed in radiation equivalents of DNA cleavage plotted against etoposide concentration on a logarithmic scale. The cultured cell lines were CCRF-CEM (△), RPMI-7666 (□), and HL-60 (▲). The freshly obtained leukemic cells from 13 patients were less sensitive and are represented by the *hatched area* on the *right*

strates the range of sensitivity exhibited by the various cells studied. Cultured cells were much more sensitive to etoposide than freshly obtained leukemic cells. There was as much as a two-log difference between the extremes. The freshly obtained leukemic cells also exhibited considerable heterogeneity with respect to drug sensitivity (Fig. 3). No clear patterns emerged with regard to diagnosis, but the number of patients was too small to allow such an analysis.

Cellular uptake of ^{3}H-etoposide

Further investigation into the causes of the observed variations in etoposide sensitivity included the determination of cellular ^{3}H-etoposide content. Figure 4 demonstrates the range of values observed among the various cell types; although there was variability with respect to content (roughly fivefold in the cultured cells at both 10 and 50 μM ^{3}H-etoposide, and three- to fourfold in fresh leukemic cells), the range of values in the cultured cells was very similar to that in the freshly obtained cells. Variations in DNA cleavage associated with etoposide can thus not be accounted for by differential cellular etoposide uptake.

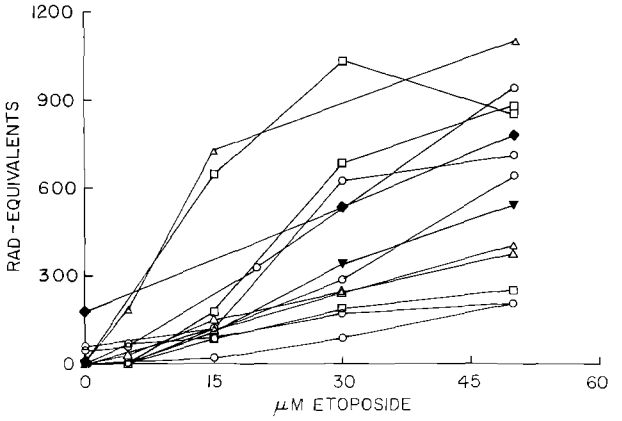

Fig. 3. Etoposide-dose response of the 13 leukemic patients studied. The data shown in Fig. 2 are shown in greater detail to demonstrate variations in etoposide sensitivity among the patients: ○, CLL; △, ALL; ◆, CML in blast crisis; □, AML; ▲, hairy cell leukemia

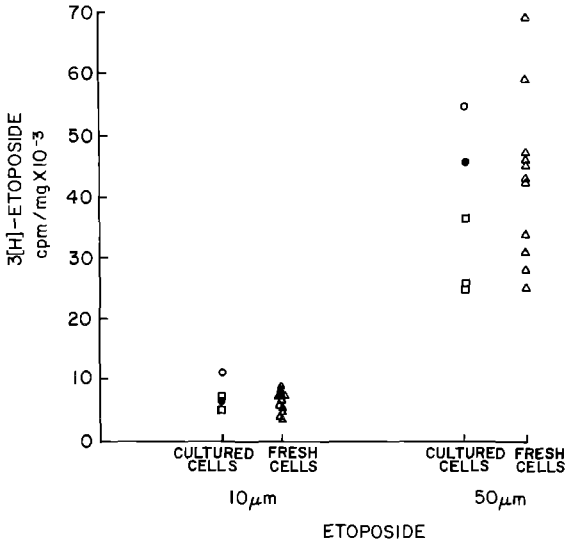

Fig. 4. Cellular uptake of ^{3}H-etoposide. Although variation is seen in amount of labeled etoposide taken up (particularly at 50 μM etoposide) there is no apparent difference between patients (△), and cell lines [CCRF-CEM (□); HL-60 (●); RPMI-7666 6○)]

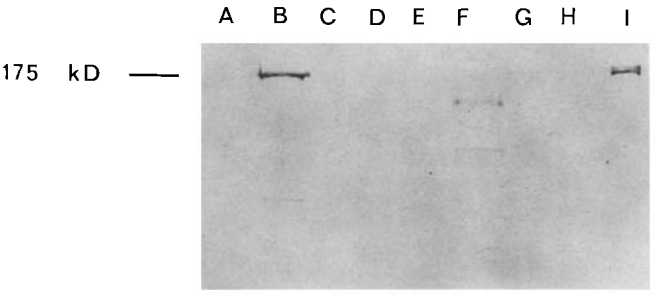

Fig. 5. Western blots of nuclear extracts. A 175-kilodalton marker is given on the *left* for reference; the *lanes* show HL-60 (*A*), CCRF-CEM (*B*), CLL patients (*C–E*), a CML patient in blast crisis (*F*), an AML patient (*G*), and an AMML patient (*H*). *Lane I* shows purified topoisomerase II from CCRF-CEM cells as a standard. Equal amounts of protein were loaded onto each lane; the CCRF-CEM preparation has the most prominent band of topoisomerase II

Whole cell protein determination

The total protein contact was determined in sonicated whole cell preparations as previously described. Not unexpectedly, the cultured cells (CCRF-CEM line) contained considerably more total protein (0.55 μg per 10^4 cells) than the freshly obtained leukemic cells. Total protein levels were determined in 5 patients with CLL (range, 0.017 μg per 10^4 cells to 0.21 μg per 10^4 cells) as well as in 1 with ALL (0.18 μg per 10^4 cells), 1 with AML (0.36 μg per 10^4 cells), and 1 with CML in blast crisis (0.15 μg per 10^4 cells).

Topoisomerase-II content

In order to determine whether differences in topoisomerase-II content might account, at least in part, for the vastly differing sensitivities of cultured and freshly obtained leukemic cells, we assayed the enzyme content of cells by immunoblotting whole cells or nuclear extracts using a mouse antibody against topoisomerase II that had been raised in our laboratory. As can be seen in Fig. 5, nuclear extracts from the CCRF-CEM cultured cell line had much greater amounts of topoisomerase II than fresh cells, even when equal amounts of protein were loaded onto the gel. Similar results were obtained when immunoblots were performed using whole cells. The fact that topoisomerase II was virtually undetectable in HL-60 cells suggested that their enzyme content is below the level of detection of our mouse serum, even though these cells display intermediate sensitivity to the DNA-cleaving effect of etoposide. The CML patient shown in Fig. 5 (lane F) exhibited two lower-molecular-weight bands; it is unknown whether these represented degradation products or otherwise altered topoisomerase II. This patient's cells, when assayed for cleavage by alkaline elution (Fig. 3), did not show unusual sensitivity to etoposide.

Influence of proliferative status

A major difference in the biologic state of the patients' cells as compared to the cultured lines was that the latter

were more proliferative. As the intracellular topoisomerase-II content is higher in proliferating cells than in quiescent cells in general, it was of interest to determine whether drug sensitivity might increase in lymphocytes that had been mitogenized into a highly proliferative state. For this purpose, we stimulated normal peripheral-blood lymphocytes with PHA and interleukin-2, and then assayed drug sensitivity only in cells which had incorporated thymidine. Figure 6 demonstrates that, under these circumstances, drug sensitivity was greatly enhanced. Indeed, the sensitivity of the stimulated lymphocytes approximated that of the CCRF-CEM line.

Western blots were performed using whole cell preparations of resting and interleukin-2-stimulated lymhocytes (Fig. 7). Topoisomerase II was only detectable in stimulated cells, although the two samples differed somewhat in intensity (compare lanes B and D).

Discussion

The data that we collected for freshly obtained human leukemia cells are consistent with previous observations that topoisomerase-II activity is an important determinant of the sensitivity of cells to DNA cleavage caused by the epipodophyllotoxin, etoposide. In fact, drug activity as measured by the alkaline elution technique can be viewed as representing a type of enzyme assay in situ, although, obviously, many other factors may influence drug action as well. A number of factors could potentially affect intracellular topoisomerase activity. Phosphorylation of this enzyme in vitro has been achieved using a casein kinase [1] as well as several oncogene-directed tyrosine kinases and protein kinase C [19, 24]. Enzyme activity is apparently stimulated upon phosphorylation. The enzyme can also be poly(ADP) ribosylated, and this inhibits its activity [5]. Conceivably, changes in intracellular location could also affect the enzyme's ability to participate in the drug-induced DNA-cleavage event. It was thus of critical importance to measure topoisomerase content. Although we cannot rule out a contributing role of post-translational modifications or intracellular compartmentalization, our data would seem to suggest that one major determinant of drug sensitivity is, in fact, enzyme content.

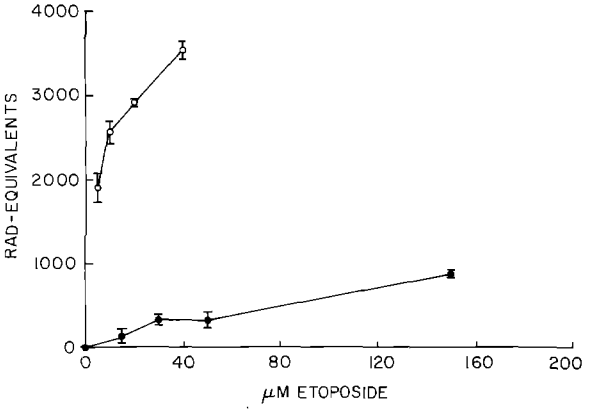

Fig. 6. Etoposide sensitivity of normal resting lymphocytes (●) and PHA IL-2-stimulated lymphocytes (○). The markedly increased sensitivity to etoposide in the stimulated cells is similar to the sensitivity of the CCRF-CEM line

The results of our study suggest that the contention of Priel's et al. [14] that leukemia cells bear an excessive amount of topoisomerase II should be reconsidered. We found that freshly obtained human leukemia cells are not significantly different than normal resting lymphocytes with respect to their sensitivity to etoposide-induced DNA cleavage, and that both types of cells are markedly less sensitive than cultured cell lines. Our data are consistent with the report of Brox et al [4] describing the DNA-cleavage activity of the topoisomerase-active agent, m-AMSA, in leukemic myeloblasts and normal lymphocytes. Our findings are also in agreement with a preliminary report by Silber et al. [20], who found CLL lymphocytes to be unresponsive to the DNA-cleaving action of adriamycin (another topoisomerase-active agent) and to have a low topoisomerase-II content as compared to HeLa cells. We believe that this difference primarily reflects the proliferative status of the cells, and that this is reflected in the effect of PHA/interleukin-2 stimulation on lymphocyte sensitivity. Tandon et al. have shown that mitogenesis increases topoisomerase-II activity in normal resting lymphocytes [23], although they did not assay the enzyme content. Our results indicate that this increase in activity is, at least partly, a function of increased enzyme content and is associated with a striking increase in drug sensitivity.

The significance of our observations should be considered in the context of the relationship between drug-induced DNA cleavage and cytotoxicity by topoisomerase-

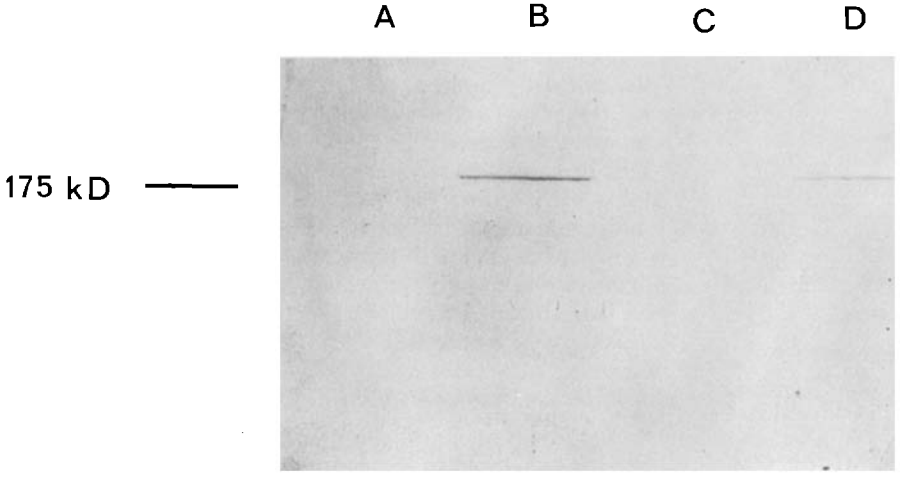

Fig. 7. Western blots of normal resting lymphocytes (lanes *A, C*) and of PHA IL-2-stimulated lymphocytes lanes (*B, D*). Volumes corresponding to 10^6 cells were loaded onto each *lane*

active agents. Three lines of evidence suggest that topoisomerase-mediated strand breaks are necessary, if not sufficient, for the expression of the antitumor effect of intercalating agents and epipodophyllotoxins. First, when closely related congeners of either class of compounds are compared, there is an excellent agreement between the potency with which they induce DNA cleavage and exert cytotoxicity [9, 18]. Second, when DNA cleavage by the epipodophyllotoxin, etoposide, is blocked using the dye, ethidium bromide, there is a striking loss of cytotoxicity as well [17]. Ethidium bromide is a unique intercalating agent in that it actually inhibits the cleavage of DNA by topoisomerase II. Third, several investigators have reported on cell lines that exhibit resistance to intercalating agents and epipodophyllotoxins which cannot be accounted for on the basis of drug uptake [2, 7, 13]. In each instance, there is a marked reduction in drug-induced DNA cleavage. Thus, while it was not possible in our study to assess sensitivity to the cytotoxic effects of etoposide, the reduction in strand-break frequency probably reflected this to some extent. Clearly, a prospective study comparing topoisomerase content, DNA cleavage, and tumor response will be necessary to confirm this point.

The observations that we made in freshly obtained human leukemia cells have potentially important clinical implications. At the very least, they suggest that topoisomerase content should be studied further as a determinant of sensitivity to epipodophyllotoxins and, very likely, intercalating agents. Interestingly, only one of our samples had a topoisomerase content sufficient high to be detected by immunoblotting. This suggests that some heterogeneity exists among human leukemia cells, although the full extent of this heterogeneity will remain unclear until a more sensitive assay for topoisomerase content is developed. A monoclonal antibody would be most helpful in this regard. It is, perhaps, not surprising that heterogeneity exists among these widely varying cell types, since there are considerable differences in the proliferation rate of these tumors. Furthermore, recent evidence obtained in our laboratory suggests that topoisomerase content is not regulated to the same extent in all cells (D. Sullivan, M Latham, and W. E. Ross, submitted for publication). We have found that, while most cells exhibited decreased topoisomerase content as they enter quiescence, in some lines, there is only a small change in content, while in others, there is no change at all. Interestingly, this appears to reflect the ability of these cells to enter a G_1-like state. Another type of heterogeneity is hinted at by the fact that the electrophoretic mobility of topoisomerase II obtained from the patient with CML in blast crisis was greater than that of CCRF lymphoblasts; however, we cannot rule out the possibility of some degree of proteolysis as a source for this phenomenon. Examination of drug-induced DNA cleavage (Fig. 3) in cells from patients also indicated considerable variability with respect to sensitivity. Unfortunately, because we were unable to detect topoisomerase content in most patients, we do not know to what extent this factor dictates differences in drug sensitivity. Finally, the observation that topoisomerase content and drug sensitivity are markedly stimulated by interleukin-2 in resting lymphocytes suggests the possibility that a similar stimulation might be observed in tumor cells if the appropriate mitogen can be identified. The observation of Zwelling et al. [27] that the estrogen-responsive breast cancer cell line, MCF-7, becomes two- to threefold more sensitive to the DNA-cleavage activity of m-AMSA following estrogen stimulation supports this hypothesis. Further work along these lines might yield potentially rewarding ways of combining biological-response modifiers and topoisomerase-active chemotherapy agents.

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