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Anthracyclines and their C-13 alcohol metabolites: growth inhibition and DNA damage following incubation with human tumor cells in culture*

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Summary. Anthracyclines are important antitumor agents used in the treatment of solid tumors, lymphomas, and acute lymphoblastic as well as myelocytic leukemias. The clinical utility of agents such as doxorubicin and daunorubicin and their well-characterized cardiotoxicity have prompted many efforts to develop analogs that retain the desired spectrum of activity but are less cardiotoxic. One such analog is idarubicin (4-demethoxydaunorubicin), which is currently under study in the treatment of adult and pediatric leukemias. The major circulating metabolite of idarubicin is the alcohol product of ketoreductase biotransformation, idarubicinol. Following the administration of idarubicin to adult or pediatric patients, systemic exposure to idarubicinol is greater than that to idarubicin. Moreover, we have also documented the presence of idarubicinol in the cerebrospinal fluid of pediatric patients who have received idarubicin. Idarubicinol has been reported to have greater cytotoxic activity than other anthracycline alcohol metabolites, which are regarded as much less active products of metabolism. We therefore evaluated the growth-inhibitory and DNA-damaging activities of idarubicin, daunorubicin, doxorubicin, epirubicin, and their alcohol metabolites against three relevant (CCRF-CEM lymphoblastic leukemia, K562 myelogenous leukemia, and U87-MG glioblastoma) human tumor cell lines. We found that whereas idarubicin was 2–5 times more potent than the other three anthracycline analogs against these tumor cell lines, idarubicinol was 16–122 times more active than the other alcohol metabolites against the same three cell lines. In addition, idarubicinol and the parent drug idarubicin were equipotent, unlike the other anthracycline alcohol metabolites, which were much less cytotoxic than the corresponding parent drugs. We also assessed the ability of the four parent drugs and their alcohol metabolites to induce DNA single-strand breaks. Idarubicin was more potent than the other three anthracycline analogs and idarubicinol was much more effective than the other alcohol metabolites in inducing DNA damage. These studies in human leukemia and human glioblastoma cell lines support the hypothesis that idarubicinol plays an important role in the antitumor activity of idarubicin and that the activities of idarubicin and idarubicinol are related to their ability to damage DNA.

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Introduction

Anthracyclines such as doxorubicin (DX) and daunorubicin (DN) are important antitumor agents used extensively in the treatment of solid tumors, lymphomas, and acute lymphoblastic as well as myelocytic leukemias [20]. The major toxicities of anthracyclines include myelosuppression, mucositis, and cardiotoxicities that are associated with the total drug dose given. Acute arrhythmias can be serious or fatal in rare circumstances, but of greatest concern is the chronic cardiomyopathy that may lead to congestive heart failure [20]. There is intense interest in the identification of anthracycline analogs that produce a higher therapeutic index, particularly with regard to cardiotoxicity, because these drugs are extremely active and because cardiotoxicity can prevent beneficial long-term therapy with these agents.

Several mechanisms of action have been proposed and extensively studied with regard to the antitumor activity of anthracyclines. These include intercalation and binding to DNA, inhibition of topoisomerase II activity, production of free radicals and other reactive species that are injurious to macromolecules within the cell, and possibly direct effects on cell membranes. Many of these mechanisms can be detected through the resultant DNA damage. Using alkaline elution techniques, both single-strand and double-strand DNA breaks have been detected following the exposure of tumor cells to a number of anthracyclines [4, 8, 25, 26, 33]. Many of these breaks are unmasked by the treatment of cell lysates with proteinase K, consistent with the

formation of protein DNA adducts, most likely through interactions with topoisomerase II [25].

4-Demethoxydaunorubicin (idarubicin, ID) is an anthracycline analog currently under evaluation for the treatment of adult and pediatric leukemias. Clinical interest in this analog is based on its greater cytotoxicity against animal [14, 28] and human [15, 27, 31] tumor cells in culture as compared with other anthracyclines. For example, ID has been reported to be 27–100 times more toxic than DN against HeLa cells [31] and to show greater activity than DN or DX against rat glioma cells in culture [28]. In preliminary studies, we found that the IC_{50} values (concentration required to inhibit the growth of 50% of the treated cells vs the control value) for ID were 2–4 times lower than those for DN against human lymphoblastic and myelogenous leukemia cells in culture [23]. ID was also more active in vivo against murine L1210 and P-388 tumors as compared with DN and DX [1, 11]. Idarubicin may be less cardiotoxic than anthracyclines such as DN and DX as judged from data obtained in several animal studies [9, 10].

We became interested in ID through evaluation of the drug for the treatment of pediatric patients with relapsed lymphocytic and myelogenous leukemia. High concentrations of the alcohol metabolite idarubicinol (IDOL) were found in plasma following intravenous administration of ID [24]. We detected IDOL in cerebrospinal fluid (CSF) at approximately 24 h after the administration of the parent drug to pediatric patients [24]. IDOL had been shown to be growth-inhibitory against tumor cells in culture [6, 12, 15, 18, 29]. Comparisons of IDOL growth-inhibition data [6, 12, 15, 29] with data reported for other anthracycline alcohol metabolites such as daunorubicinol (DNOL) and doxorubicinol (DXOL) [21, 23, 29, 34] suggested that IDOL was more potent than the other alcohols.

Conversion of parent anthracyclines to their alcohol metabolites via ketoreductase metabolism is generally regarded as an inactivation pathway for anthracyclines on the basis of the relatively lower activity of the corresponding alcohol metabolites. We believe that this supposition is incorrect for IDOL, given its growth-inhibitory activity, its high concentrations in plasma following administration of the parent drug [24, 30, 35], and its presence in CSF after the administration of ID [24]. We wished to assess carefully the growth inhibition produced by structurally related anthracyclines, including ID, and their alcohol metabolites in a single study using relevant human tumor cell lines and to assess the resultant DNA damage for comparison with the growth-inhibition data. This report describes the results obtained in human lymphoblastic (CCRF-CEM) and myelogenous (K562) leukemia cell lines and in a human glioblastoma (U87-MG) cell line.

Materials and methods

Drugs. ID, IDOL, DNOL, epirubicin (EP), epirubicinol (EPOL), and DXOL were generously provided by Farmitalia (Milan, Italy) and Adria Laboratories (Columbus, Ohio). All alcohols were of the C-13 (S) configuration obtained via microbial reduction [22]. DX and DN were purchased from Sigma Chemical Company (St. Louis, Mo.). Stock solutions of the drugs were prepared in sterile saline and stored in a dark environment at –20° C. Drug stocks were diluted with saline just prior to their use.

Cell cultures. CCRF-CEM human acute lymphoblastic leukemia cells, K562 human chronic myelogenous leukemia cells, and U87-MG human glioblastoma astrocytoma cells were obtained from the American Type Culture Collection (Rockville, Md.). CCRF-CEM and K562 cells grown in suspension were maintained in RPMI media containing 20% and 10% fetal calf serum, respectively. U87-MG cells were maintained as a monolayer culture in MEM media containing Earle's salts, 0.1 mM nonessential amino acids, 0.1 mM sodium pyruvate, and 10% fetal calf serum. All cultures were incubated at 37° C in an atmosphere containing 5% CO_2, and all experiments were performed using cells in logarithmic growth.

Growth-inhibition assay. CCRF-CEM and K562 cells were seeded at 5×10^4 cells/ml in 25-cm^2 tissue-culture flasks. U87-MG cells were seeded at 1×10^5 cells/60-mm tissue-culture dish. Drug stocks were thawed and diluted in saline just prior to their addition to the media. Cells were exposed to drugs for 1–72 h. When appropriate, drug and media were replaced every 24 h due to the gradual decomposition of some anthracyclines under incubation conditions. Cell suspensions were centrifuged, the cell pellet was taken up in fresh media, and a new aliquot of drug was added. For monolayer cultures, medium was removed by suction and fresh drug-containing medium was added. At the end of the exposure period, an aliquot of cell suspension was removed from the flasks for counting. The monolayer cultures were treated briefly with trypsin before an aliquot of cells was taken from the dishes for counting. Cell counting was done on a Coulter counter and the results were expressed as percentages of inhibition relative to control (no drug) values.

Two flasks or dishes per concentration of drug were run per experiment against three control flasks or dishes. Each experiment was repeated at least two to three times. IC_{50} values were then calculated from these experimental data as the concentration of drug required to cause 50% inhibition of growth of the treated cells as compared with control values over a 72-h incubation period.

Alkaline elution. Protein-associated DNA single-strand breaks were assessed by the procedure of Kohn et al. [17]. The cells were labeled with 0.02 μCi methyl-[^{14}C]-thymidine/ml (0.36 nmol/ml) or 0.01 μCi methyl-[^{3}H]-thymidine/ml (0.91 nmol/ml) for 48 h in 25-cm^2 flasks at concentrations of $2.5–5 \times 10^5$ cells/ml in 10 ml media. This allows 1.5–2 cell doublings. For the slower growing monolayer cultures (U87-MG), cells were exposed to label for 24 h followed by a 24-h chase period. Stock solutions of drug were thawed and diluted with normal saline just prior to their addition to the cells. The cells were exposed to drug for 1 h and then washed (U87-MG cells being briefly trypsinized), and 5×10^5 cells were placed on 2-μm polyvinylchloride filters. Internal-standard cells were subjected to 150 rad from a cesium 137 source, and 5×10^5 cells were placed on each filter along with the experimental cells. The cells were subjected to lysis using a solution of 2% sodium dodecyl sulfate (SDS) and 0.02 M ethylenediaminetetraacetic acid (EDTA, pH 10) followed by proteinase K (0.5 mg/ml lysis solution) digestion prior to the 15-h elution with 0.02 M EDTA/tetrapropylammonium hydroxide buffer (pH 12.1). The single-strand-break frequency (P_{BD}) was calculated using the following equation [32]:

$$P_{BD} = \frac{\log (r_1/r_o)}{(R_o/r_o)} P_{BR},$$

where P_{BR} represents the DNA break frequency produced by X-irradiation (400 rad) and r_1, r_o, and R_o represent the retention of DNA from drug-treated, untreated, and irradiated cells, respectively. Retention was evaluated when 60% of the internal-standard DNA remained on the filter.

High-performance liquid chromatography. Determination of parent anthracyclines and alcohol metabolites was carried out using a modification of the reverse-phase high-performance liquid chromatographic (HPLC) procedure previously employed in our laboratory [24]. Anthracyclines were extracted from samples by solid-phase extraction on Baker C_{18} columns, chromatographed on an IBM C_{18} column, and detected by fluorescence (excitation wavelength, 254 nm; emission wavelength, >470 nm).

Table 1. Growth inhibition induced in human tumor cells following 72 h exposure to anthracyclines and their alcohol metabolites

Agent	IC_{50} (nM)		
	CCRF-CEM cells	K562 cells	U87-MG cells
ID	2.4± 0.5	2.7± 0.4	4.7±0.9
IDOL	1.8± 0.4	2.9± 0.6	4.7±0.7
DN	4.6± 0.5	10.0± 1.4	10.5[a]
DNOL	136.9± 9.8	85.4± 5.1	74.9[a]
DX	5.9± 1.1	9.9± 0.9	7.6±1.5
DXOL	219.9±39.1	207.9± 5.5	168.4±6.0
EP	3.5± 0.7	11.6± 0.4	4.7[a]
EPOL	182.4±27.9	303.0±20.5	221.7[a]

Data represent mean IC_{50} values ± SEM ($n = 3$)
[a] Average of 2 experiments

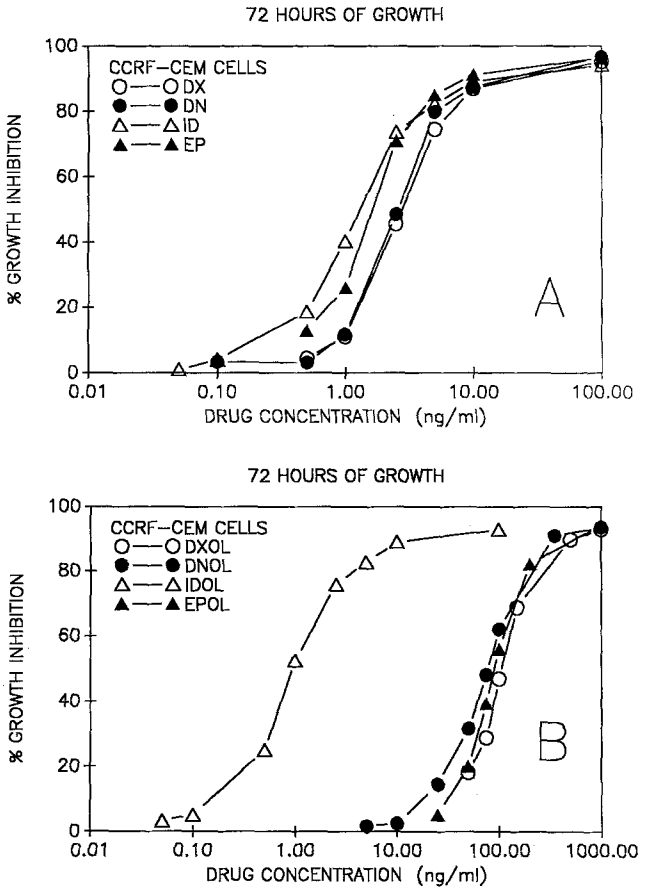

Fig. 1 A, B. Dose-response growth-inhibition curves for 72-h incubations of CCRF-CEM cells with **A** ID, DN, DX, and EP and **B** IDOL, DNOL, DXOL, and EPOL

Results

Growth inhibition

The growth-inhibitory activity of ID, DN, DX, and EP were determined following incubation of the drugs with CCRF-CEM human acute lymphoblastic leukemia cells and K562 human chronic myelogenous leukemia cells. A continuous 72-h exposure period was used for most studies due to our interest in daily ×3 administration of ID to pediatric cancer patients and to the relatively prolonged plasma elimination of anthracyclines and their alcohol metabolites. We also evaluated these drugs against U87-MG human glioblastoma cells on the basis of our previous detection of IDOL in the CSF of 19/20 pediatric patients who had received ID [24]. Half-life values (determined by HPLC analyses) determined for ID, DN, and DX were ~30, 304, and 50 h, respectively, in RPMI cell-culture media (37°C) and 35, 495, and 100 h, respectively, in MEM cell-culture media. Accordingly, drug and media were replenished every 24 h during the 72-hour incubations.

Results of the 72-h studies for both of the human leukemia cell lines and the human glioblastoma cell line are summarized in Table 1. Data obtained during incubations of CCRF-CEM cells are shown graphically in Fig. 1 A. Based on IC_{50} values, ID was approximately 4 times more potent than the other three analogs against the myelogenous K562 cells. ID and EP were approximately twice as potent as DN and DX against the lymphoblastic CCRF-CEM cells. ID and EP were equipotent against the U87-MG glioblastoma cells and approximately 2 times more potent than DN or DX. We assessed the conversion of the parent drugs to their alcohol metabolites during 72-h incubations of the former with cells. HPLC analyses revealed that less than 5% of the ID, DN, and DX was converted to the corresponding alcohols metabolite during 72-hour incubations with K562 and CCRF-CEM leukemia cells. The glioblastoma cells converted approximately 10% of the ID and 20% of the DN to the respective alcohol metabolites during the 72-h incubation periods.

The growth-inhibitory activity of the alcohol metabolites IDOL, DNOL, DXOL, and EPOL were determined against the three cell lines under the same experimental conditions used for the parent drugs. Half-life values for the alcohols in cell-culture media were greater than 100 h. The striking growth inhibition of IDOL as compared with the other three anthracycline alcohol metabolites following exposure of the two human leukemia cell lines and the human glioblastoma cell line to ID is reflected in the IC_{50} values summarized in Table 1. Figure 1 B illustrates the data obtained during incubations of CCRF-CEM cells. Based on IC_{50} values, IDOL was 29–104 times more active than the other alcohol metabolites against K562 cells, 76–122 times more potent against the CCRF-CEM cells, and 16–47 times more potent against the U87-MG cells. In contrast, the IC_{50} values obtained for DNOL, DXOL, and EPOL showed less than a 3-fold variation between the two cell lines.

Of particular interest was the relationship between the growth inhibition produced by the parent drugs and that caused by the corresponding alcohol metabolites. As shown by the IC_{50} ratios of parent drug/alcohol metabolite listed in Table 2, IDOL and ID were essentially equipotent against all three human tumor cell lines (Table 1). DNOL, DXOL, and EPOL were 7–52 times less active than the respective parent drugs against these cell lines.

Table 2. Ratios of growth inhibition determined for parent drugs and their alcohol metabolites following treatment of human tumor cells

Agent	IC_{50} ratio[a]		
	CCRF-CEM cells	K562 cells	U87-MG cells
ID/IDOL	1.33	0.93	1.0
DN/DNOL	0.03	0.12	0.14
DX/DXOL	0.03	0.05	0.05
EP/EPOL	0.02	0.04	0.02

[a] IC_{50} value found for each parent drug divided by that obtained for its alcohol metabolite

Table 3. Relative growth inhibition and DNA damage induced by anthracyclines and their alcohol metabolites in CCRF-CEM cells

Agent	Relative growth inhibition[a]	Relative DNA damage[b]
Parent drug:		
ID	100	100
DN	52.2	14.3
DX	40.7	2.1
EP	68.6	9.3
Alcohol metabolite:		
IDOL	100	100
DNOL	1.3	3.3
DXOL	0.8	0.1
EPOL	1.0	0.1

[a] Growth inhibition as based on IC_{50} values and expressed as a percentage relative to ID for parent drugs or to IDOL for alcohol metabolites
[b] DNA damage as calculated from the initial linear slope of DNA SSB frequency-concentration curves and expressed as a percentage relative to ID for parent drugs and to IDOL for alcohol metabolites

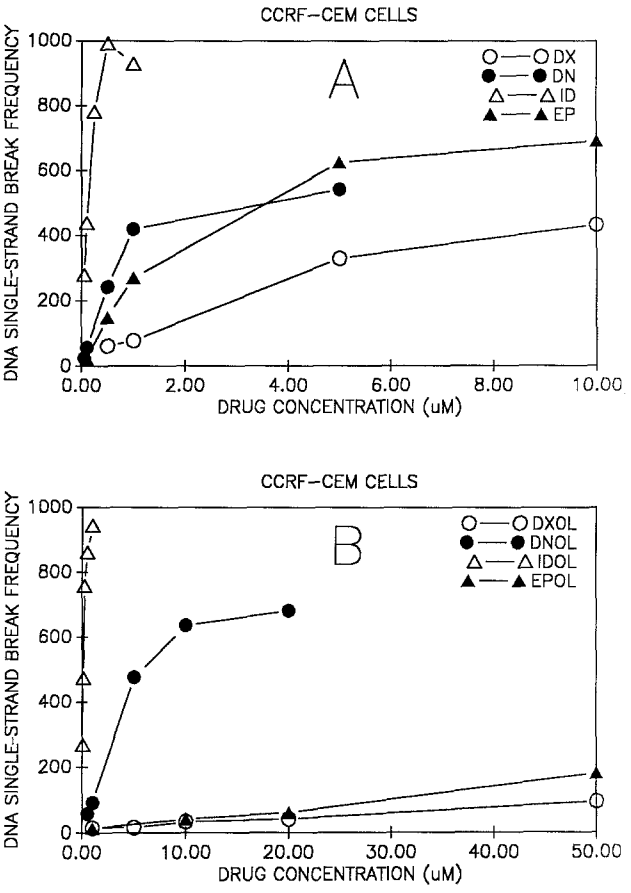

Fig. 2A, B. Protein-associated DNA SSB produced following a 1-h exposure of CCRF-CEM cells to **A** ID, DN, DX, and EP and **B** IDOL, DNOL, DXOL, and EPOL. The SSB frequency was calculated using the retention end points in the elution when 60% of the internal-standard DNA remained on the filter

Studies of DNA damage

In an effort to characterize further the differences in the growth inhibition produced by these eight molecules, particularly the striking activity of IDOL, DNA single-strand breaks (SSB) were assessed following the incubation of tumor cells with these agents. We elected to evaluate SSB by alkaline elution after proteinase K treatment to reveal protein-associated as well as non-protein-associated SSB. Data obtained for the four parent drugs following incubation with CCRF-CEM cells are shown graphically in Fig. 2A. ID was the most active inducer of DNA SSB and DX was the least potent. The same order of SSB activity was observed for the alcohol metabolites (Fig. 2B), with DXOL and EPOL being very poor inducers of DNA SSB as compared with DNOL and, particularly, IDOL. When ID, IDOL, DN, and DNOL were evaluated following incubations with K562 cells, DNA SSB were similar to those observed in the CCRF-CEM cells (data not shown). We also characterized DNA SSB in the U87-MG cell line following incubations with DX, DXOL, ID, and IDOL. ID and IDOL were the most active inducers of DNA SSB damage in this cell line (data not shown).

For comparative purposes, we calculated the initial linear ($r^2 \geq 0.93$) slopes of the DNA SSB frequency-concentration curves shown for CCRF-CEM cells in Figs. 2A and 2B. These values were then used as a measure of DNA damage for comparison with the growth-inhibition (IC_{50}) data obtained for the four anthracyclines and the four alcohol metabolites (Table 3). Although ID was only 1–2 times more potent than the other three parent drugs with regard to growth inhibition, it was a much more potent inducer of DNA damage than the other analogs. In contrast, the strikingly greater cytotoxicity of IDOL as compared with the other three alcohols was reflected in the DNA SSB data. We also calculated the slope ratios of alcohol to parent drug for IDOL/ID, DNOL/DN, DXOL/DX, and EPOL/EP, obtaining values of 0.97, 0.23, 0.03, and 0.01, respectively. Comparison of these values with the growth-inhibition ratios shown in Table 2 for CCRF-CEM cell studies revealed a high degree of correlation between alcohol/parent drug ratios of DNA damage and growth inhibition for three pairs of parent drugs and metabolites but not for DNOL and DN. The DNA SSB activity of DNOL relative to DN was greater than that predicted by the ratio of IC_{50} values for DNOL and DN.

Discussion

A number of investigators have published growth-inhibition data for anthracycline analogs and, to a lesser extent, for their alcohol metabolites. A direct comparison of selected analogs and alcohol metabolites has been difficult because most studies have focused on only one or two parent drugs and/or alcohol metabolites and a variety of cell lines (often not of human origin) and assay conditions have been used in such studies. We characterized the growth-inhibitory activity of ID and IDOL and compared the results with those obtained for three structurally related anthracyclines and their alcohol metabolites using three human tumor cell lines for all studies. The CCRF-CEM lymphoblastic leukemia and K562 myelogenous leukemia cell lines were selected because of the extensive use of anthracyclines, including ID, in the treatment of acute lymphoblastic and myelogenous leukemias. The U87-MG glioblastoma cell line was selected on the basis of our recent finding that IDOL was consistently present in the CSF of pediatric patients receiving ID in a phase I leukemia study [24]. Anthracyclines are not widely thought to penetrate the blood-brain barrier and are not used in the treatment of CNS disease.

Based on the IC_{50} values shown in Table 1, ID was overall the most effective inhibitor of tumor cell growth among the four anthracycline analogs tested. However, EP was as effective as ID against the U87-MG and CCRF-CEM cell lines. Although it is often noted that ID is far more toxic than DN or DX, the data we obtained in human leukemia and glioblastoma cell lines included IC_{50} values that were no more than 2–5 times lower for ID as compared with DN, DX, and EP. Similar results have been reported for 2-h incubations with rat C_6 glioblastoma cells. In that study, ID was the most potent anthracycline, yielding IC_{50} values that were 3–12 times lower than those obtained for DN, DX, or EP [28]. We detected minimal conversion (<5%) of parent anthracyclines to alcohol metabolites during 72-h incubations with the human leukemia cell lines. However, the human glioblastoma cells converted higher amounts of ID (~10%) and DN (~20%) to their respective alcohol metabolites. Although there are several reports of tumor cell metabolism of anthracyclines to alcohol metabolites, the percentage of conversion reported has most frequently been 10% or less [3, 4, 7, 13, 16]. However, these studies used incubation periods of 1–4 h, whereas those in the present study lasted for 72 h. The conversion of DN to DNOL (~20%) may have resulted in a modest underestimation of DN growth-inhibitory activity following incubations with U87-MG cells due to the low potency of DNOL. Since IDOL and ID were essentially equipotent against all three cell lines, conversion of 10% of the ID to IDOL had little impact on the characterization of ID growth inhibition in the glioblastoma cells.

In contrast to the results obtained using ID, IDOL was much more active than DNOL, DXOL, or EPOL against all three human cell lines, similar to the findings previously reported for the four alcohols following 2-h incubations with rat C_6 glioblastoma cells [29]. Most interesting was the comparison of the parent drug/alcohol metabolite IC_{50} values shown as ratios in Table 2. IDOL was unique in that its growth-inhibitory activity was equal to that of the parent drug. The alcohols used in these studies were of the C-13 S configuration, as was the alcohol previously recovered in urine from ID-treated patients [5].

Ketoreductase conversion of anthracyclines to their alcohol metabolites, which is more efficient for more lipophilic anthracyclines such as DN and ID [2, 19], is primarily regarded as an inactivation pathway for anthracycline drug elimination. This is most likely true for DX, DN, and EP, given that their alcohol metabolites are much less active inhibitors of cell growth as compared with the parent drugs. In vitro data from this study as well as data from other investigations [6, 12, 18, 29] are consistent with a role for IDOL in the antitumor activity and, possibly, the toxicity of ID. This is particularly true since systemic exposure to IDOL is much greater than that to ID in both adult and pediatric patients following ID administration [24, 30, 35] and because the elimination of IDOL is greatly prolonged as compared with that of the parent drug. Plasma concentrations of IDOL lie well above the growth-inhibitory values (based on IC_{50} values for a 72-h exposure period) for more than 3 days after either single-dose or daily $\times 3$ administration of ID [24, 30, 35]. It is more difficult to speculate about the relevance of IDOL in the treatment of CNS disease. Mean CSF concentrations of IDOL measured at 24 h after the administration of 10–12.5 mg/m^2 ID were ~0.5 ng/ml [24], which is a marginally growth-inhibitory concentration for a 72-h exposure period. However, the prolonged plasma elimination of IDOL and the possibility that higher CSF concentrations might occur at earlier times following ID administration may mean that the total exposure of cells in the CNS to IDOL affects tumor growth. Studies are currently under way to characterize further the CSF concentration-time profile of ID and IDOL in pediatric patients.

DNA damage is associated with a number of potential anthracycline mechanisms of action. Anthracyclines have been shown to induce DNA SSB and double-strand breaks (DSB) associated with the formation of DNA topoisomerase II-drug complexes [25, 33]. We chose to assess total SSB because some anthracyclines induce non-protein-associated SSB as well as protein-associated SSB [4]. ID and IDOL were by far the most potent inducers of DNA damage as compared with the other anthracyclines and their alcohol metabolites (Fig. 2, Table 3). In some manner, we wished to compare growth inhibition (IC_{50} values) with DNA damage as determined by measurements of DNA SSB. We elected to rank DNA SSB activity by determining the initial slope of the DNA SSB frequency-concentration curves. The nonlinear relationships observed at high concentrations (proposed by some investigators as being related to DNA distortion, which reduces the ability of cells to form DNA topoisomerase-dependent SSB [4]) were of less interest for the purposes of comparison with growth inhibition.

For the parent drugs, DNA damage did not correlate particularly well with growth inhibition. For example, although ID was only twice as potent as DN and DX in inhibiting the growth of CCRF-CEM cells, it was 7–50 times more active as the other three analogs in inducing

DNA SSB (Table 3). Similar findings were noted for K-562 and U87-MG cells (data not shown). The DNA damage induced by alcohol metabolites did correlate well with growth inhibition. IDOL was by far the most effective inhibitor of growth and the most effective inducer of DNA SSB following incubation with CCRF-CEM cells (Table 3). IDOL data were similar for K-562 and U87-MG cells (data not shown). The relationships observed between growth inhibition and intracellular effects such as DNA damage may have been due to a lesser or greater extent of uptake of the molecules by tumor cells. In preliminary studies, we have found that uptake of the parent drug correlates relatively well with growth inhibition but not as well with DNA damage; uptake accounts less adequately for the unique activity of IDOL as compared with the other alcohol metabolites (Ames et al., unpublished data).

Finally, we considered alcohol/parent drug relationships with regard to growth inhibition (reflected by the IC_{50} ratios shown in Table 2) and DNA SSB. Comparison of the slope ratios reported in Results with the IC_{50} ratios listed in Table 2 for CCRF-CEM studies revealed that IDOL is uniquely active as compared with the parent drug ID in terms of both growth inhibition and DNA damage. IDOL has recently been shown to induce DNA DSB in a manner similar that displayed by ID following incubation with a patient-derived leukemia cell line [18]. Although these data do not address the mechanisms involved, they do suggest that the unique growth-inhibitory activity of IDOL may be related to mechanisms that damage DNA.

This study presented comparative data on growth inhibition and one measure of DNA damage obtained for ID and three structurally related anthracyclines as well as their alcohol metabolites using three human tumor cell lines relevant to current and potential clinical applications of ID. We confirmed the growth-inhibitory activity previously reported for ID and IDOL in several human tumors and a number of animal models [4, 6, 12, 28, 29]. In direct comparison with the three cell lines, we showed that IDOL and ID are equipotent inhibitors of cell growth. Although we used an arbitrary measure of DNA damage, the unique behavior of IDOL was reflected in the DNA SSB data obtained. Our interest continues in the basis of the unique pharmacology of IDOL in comparison with the alcohol metabolites of the other anthracyclines and in its unique activity as compared with that of the parent drug.

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