Enhanced Sensitivity of the Rat Hepatoma Cell to the Daunorubicin Analogue 4-Demethoxydaunorubicin Associated with Induction of DNA Damage

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ABSTRACT

The H-35 rat hepatoma, a cell line which is relatively resistant to the classical anthracycline antibiotics such as Adriamycin [the concentration of drug which inhibits cell proliferation by 50% (IC50) = 2.5 µM] and daunorubicin (IC50 of 0.5 µM), is markedly more sensitive to the 4-demethoxydaunorubicin derivative, idarubicin (IC50 of 0.025 µM). In contrast to daunorubicin, which has previously been shown to inhibit hepatoma cell proliferation in the absence of perceivable DNA cleavage, idarubicin induces concentration-dependent DNA damage which may account for its enhanced capacity to inhibit proliferation of the rat hepatoma. Free radical scavengers fail to interfere with inhibition of cell proliferation induced by idarubicin. Damage to the cell membrane or alterations in mitochondrial integrity do not appear to represent components of idarubicin toxicity in this tumor cell line. Inhibition of DNA synthesis by idarubicin parallels inhibition of cell growth; however, sensitivity of DNA synthesis to idarubicin is significantly less than that for cell proliferation (IC50 values of 0.5 µM and 0.025 µM, respectively). It is postulated that the antiproliferative effects of idarubicin in the H-35 rat hepatoma model may be a consequence of alterations in DNA integrity which ultimately result in the inhibition of cellular biosynthetic processes.

INTRODUCTION

The anthracycline antibiotics, Adriamycin and daunorubicin, have been utilized extensively in the treatment of leukemias, lymphomas and, in the case of Adriamycin, solid tumors in humans (1). However, the clinical utility of these agents is limited by their cumulative myocardial toxicity (2). Consequently, anthracycline analogues, such as 4-demethoxydaunorubicin (idarubicin) have been synthesized with the goal of dissociating antitumor activity from cardiotoxicity (3).

Idarubicin has several distinct advantages over ADR and DNR. The 4-demethoxy derivative exhibits activity not only by i.v. infusion, but following oral administration as well (4). The therapeutic index of IDR is superior to that of Adriamycin and daunorubicin (5) and IDR demonstrates an enhanced antitumor effect in anthracycline-resistant tumor cell lines (6).

Although the anthracycline antibiotics and their analogues, including 4-demethoxydaunorubicin (idarubicin), have undergone extensive preclinical and clinical studies (4, 7-9) over the past decades, an unequivocal biochemical characterization of their mechanism of action has not been established. This is due, in part, to the molecular complexity of the anthracyclines and the multiple mechanisms which may be involved in cell injury mediated by these agents.

The anthracyclines bind to DNA primarily by intercalation (10, 11), effectively deforming the three-dimensional structure of the DNA molecule and diminishing the activity of DNA and RNA polymerases, with resultant inhibition of DNA and RNA synthesis (12). The anthracyclines have been shown to produce enzyme-mediated and/or iron-mediated highly reactive free radicals in Ehrlich ascites (13) and MCF-7 breast tumor cells (14). Direct association of drug with the cellular membrane has also been proposed to account for the toxicity of the anthracycline antibiotics (15). However, the primary mechanism of action of the anthracyclines is generally thought to be to the formation of "DNA-protein complexes," which give rise to protein-associated strand breaks in DNA upon enzymatic deproteinization (16-18). These labile "cleavable-complexes" are thought to arise as a consequence of drug interference with the DNA breakage and rejoicing actions of mammalian Topoisomerase II (16-18), an enzyme which facilitates DNA segregation and chromosomal dysjunction (19).

The anthracycline antibiotic, Adriamycin, is utilized with limited success in the treatment of hepatocellular carcinoma (20). In order to elucidate the basis for the relative unresponsiveness of primary hepatocellular carcinomas to chemotherapy with anthracyline antibiotics, our laboratory has studied the interaction of these agents with the H-35 rat hepatoma, a cell line which has been shown to be quite insensitive to the anthracycline antibiotics both in vitro and in vivo (21, 22). In a previous report, we demonstrated limited induction of DNA damage by daunorubicin in the H-35 rat hepatoma cell (23). The studies presented in this report indicate that enhanced sensitivity of the hepatoma cell to idarubicin as compared to daunorubicin may be associated with the capacity of the 4-demethoxy-daunorubicin analogue to induce DNA damage in this tumor cell line.

MATERIALS AND METHODS

Materials

IDR was kindly provided by Adria Laboratories (Columbus, Ohio). Stock solutions of IDR were prepared in filtered methanol and drug concentrations determined spectrophotometrically (at a 1:50 dilution) assuming a maximum absorbance of 2.17 for a 100 µM solution in methanol at 483 nm as indicated by Pharmatia Laboratories, Italy. Exposure of drug solutions to light was avoided.

Doxorubicin hydrochloride, daunorubicin hydrochloride, MTT for cell proliferation and mitochondrial function assays, bisbenzimid trihydrochloride (Hoechst dye 32258), and reagents for the GOT assay were obtained from Sigma Chemical Co., St. Louis, MO. Tetrapropyrammonium hydroxide for the alkaline elution assay was obtained from Eastman Kodak Co., Rochester, NY. All other chemicals were reagent grade. DNA, RNA, and protein synthesis were monitored utilizing [methy-3H]thymidine (61 Ci/mol), [5-3H]uridine (18 Ci/mol), and 4,5-3H-l-leucine (50 Ci/mol) from ICN Radiochemicals, Irvine, CA.

Cell Culture Conditions

H-35 rat hepatoma cells (H-4-II-E) were obtained from the American Type Culture Collection (Rockville, MD) and maintained as a monolayer culture in "complete medium." Medium was composed of Dulbecco's minimal essential medium (Hazelton Research Products, Denver, PA) supplemented with 5% fetal calf serum (Life Technologies,
Grand Island, NY), 5% defined bovine calf serum (HyClone Laboratories, Logan, Utah), glutamine (29.2 mg/100 ml), and penicillin/streptomycin (0.5 mg/100 ml). Cells were incubated at 37°C, under 5% CO₂, and all assays were performed with cells in logarithmic growth.

Methods

Drug Effects on Cell Proliferation. Cells in log growth were incubated with varying concentrations of idarubicin for a 2-h period. Seventy-two h later, proliferation was monitored using the MTT tetrazolium dye assay as described previously (23). Following a 3-h incubation with MTT, dye was extracted in DMSO and dye absorbance was quantitated spectrophotometrically using a Model EL310 EIA autoreader at 540 nm.

Cell Cycle Analysis. Hepatoma cells in log growth were treated with propidium iodide staining solution consisting of 3.8 mM sodium citrate, 0.1% Triton X-100, RNase B (7000 units/ml), and 0.05 mg propidium iodide/ml and refrigerated for 30 min (24). Cells were dislodged using a rubber policeman. The stained nuclei were analyzed for DNA-propidium iodide fluorescence using a Coulter Teletronics TPS-I at a laser setting of 36 mw and an excitation wavelength of 488 nm; resulting DNA distributions were analyzed for the proportion of cells in the G-, S-, and G2-M phases of the cell cycle as described by Collins et al. (25).

Influence of Free Radical Scavengers on Cell Sensitivity to Idarubicin. Cellular proliferation assays were performed using the methodology described above. Prior to the 2-h drug incubation with idarubicin in the presence of free radical scavengers or deferoxamine, cells were incubated for a period of 1 h with free-radical scavengers at twice the indicated final concentrations: Catalase 1,500 units/ml, DMSO 0.5 μM, mannitol 50 mM, methanol 150 mM, or the iron chelator, deferoxamine at 0.5 × 10⁻² M.

Alkaline Unwinding Assay for DNA Cleavage. The alkaline unwinding assay for monitoring induction of DNA strand breaks, was performed as described by Kanter and Schwartz (26) where DNA damage is monitored based on the differential binding of Hoechst dye to single- and double-stranded DNA. H-35 cells were incubated with varying concentrations of IDR for a 2-h period. Cells were washed with warm phosphate buffered saline (0.15 M NaCl buffered to pH 7.4 with mono and dibasic potassium phosphate), released from culture flasks utilizing trypsin (0.05 mg/ml) and EDTA (0.02 mg/ml) in saline, and resuspended to a density of 1 × 10⁶ cells/ml. An unwinding period (in alkalai) of 10 min was utilized, and fluorescence of the Hoechst dye-DNA complex was determined after elution from a Kratos fluorescence spectrophotometer with an excitation wavelength of 350 nm and emission at 450 nm. DNA damage was quantitated by comparison to that induced by X-irradiation.

Alkaline Elution. The alkaline elution assay was utilized to discriminate between direct and protein-associated DNA strand breaks. H-35 rat hepatoma cells were plated in 75-cm² canted-neck culture flasks (Costar, Boston, MA) at a density of 1 × 10⁶ cells/ml at least 72 h prior to initiation of the study. Cells were incubated for 24 h in 12-15 ml of complete medium containing 0.2 μCi/nmol of [³H]thymidine followed by a 24-h incubation in [³H]thymidine-free medium to permit short fragments of labelled DNA to be incorporated into complete strands.

Cells were treated for 2 h with various concentrations of idarubicin in complete medium. After two washes with phosphate buffered saline, cells were detached from flasks by incubation with 5 ml of trypsin (0.05 mg + EDTA (0.02 mg/ml saline) at 37°C under 5% CO₂ for 5 min. Cells in the trypsin solution were diluted 1:10 with cold phosphate buffered saline or complete medium to prevent protelysis and to limit repair of DNA strand breaks. Cell suspensions were pelleted at 500 × g in a refrigerated centrifuge and resuspended in cold phosphate buffered saline on ice. The alkaline elution procedure utilized is a modification (23) of that described by Kohn et al. (27, 28). Approximately 1.5 × 10⁶ cells were lysed on protein retentive 0.8-μM polyvinyl chloride filters (Gelman Sciences, Ann Arbor, MI) with 0.2% N-lauryl sarcosine/2 M NaCl/0.04 M EDTA (pH 9.7) at room temperature for 30 min. Proteinase K (0.5 mg/ml in lysis solution) was added where appropriate to allow expression of protein-associated DNA damage. DNA was eluted using tetrapropylammonium hydroxide (pH 12.1) at a flow rate of 25–40 μl per minute. Fractions were collected over a 17-h period and analyzed by liquid scintillation counting; [³H]-DNA associated with the filter and in cell lysates was quantitated as well.

Double-strand breaks in DNA were monitored by neutral elution. Cells lysed with 2% sodium dodecyl sulfate, 0.02 M EDTA (pH 10) on polycarbonate filters (Nucleopore) in the presence of proteinase K were eluted using tetrapropyl ammonium hydroxide at pH 9.6.

Accumulation and Retention of Daunorubicin and Idarubicin. Cells were incubated with either daunorubicin or idarubicin (0.5 μM) for a 2-h period. For the accumulation studies, cells were washed with cold phosphate buffered saline and scraped gently into the saline solution. An aliquot of the cell suspension was reserved for protein determination by the method of Bradford (29). For drug retention studies, cells were processed similarly following a 2-h incubation in drug-free medium. Drugs were extracted into chloroform/methanol (2:1, v:v), concentrated to dryness and reconstituted in methanol. Drug fluorescence was determined after elution from a reversed-phase column (30) using an excitation wavelength of 480 nm and emission wavelength of 580 nm. This method of analysis permitted accurate determination of low levels of drug fluorescence. Cellular levels of daunorubicin and idarubicin were quantitated using standards of known concentration, and normalized for cellular protein content.

To assess cell viability immediately following exposure to drug, H-35 cells were incubated with varying concentrations of IDR for a 2-h period and viable cells determined by Trypan blue exclusion using a standard Neubauer hemocytometer and light microscope. Assays for drug effects on macromolecular biosynthesis, mitochondrial function, and GOT leakage were performed as described previously (23).

RESULTS

Sensitivity of Cellular Proliferation to Idarubicin, Daunorubicin, and Adriamycin. Fig. 1 demonstrates the relative insensitivity of the H-35 rat hepatoma to growth inhibition by daunorubicin and Adriamycin, and the more pronounced sensitivity to idarubicin (over a range of drug concentrations between 0.01

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3 It is important to note that lysis of large numbers of hepatoma cells on polyvinyl chloride filters appears to prevent filter binding of the Topoisomerase II DNA complex, resulting in a lack of discrimination between protein-associated and nonprotein-associated DNA strand breaks in the alkaline elution assay. (Gewirtz, D. A., Ellis, A. L., Randolph, J. K., Yanovitch, S., Swerdlow, P. S., Povirk, L. F. and Yalowich, J. C. Expression of protein-associated DNA damage in the alkaline elution assay in the absence of enzymatic deproteinization. Submitted for publication.)

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Fig. 1. Drug sensitivity of H-35 rat hepatoma. H-35 rat hepatoma cells in logarithmic growth were incubated with the appropriate drug at concentrations between 0.01 and 5.0 μM for a period of 2 h. Number of viable cells was determined using the MTT tetrazolium dye assay. Data are presented as mean values ± standard errors for 20–25 individual experiments.
IDARUBICIN-INDUCED DNA DAMAGE IN RAT HEPATOMA

100
80
60
40
20

% INHIBITION OF SYNTHESIS

[|DATURUCIN], nM

DNA
RNA
PROTEIN

0.0 0.2 0.4 0.6 0.8 1.0 1.2

Fig. 2. Effect of idarubicin on macromolecular biosynthesis. H-35 rat hepatoma cells in log growth were incubated with idarubicin at concentrations between 0.01 and 1.0 μM for 2 h. Rates of DNA, RNA, and protein synthesis are presented as mean values ± standard errors for five to nine individual experiments.

and 5.0 μM). The IC50 for growth inhibition by daunorubicin is approximately 0.5 μM,4 and for Adriamycin 2.5 μM, while for idarubicin, the IC50 is 0.025 μM. At a clinically relevant concentration, such as 0.1 μM (31, 32), daunorubicin and Adriamycin inhibit proliferation of the H-35 rat hepatoma by less than 30%, while idarubicin inhibits growth by 83 ± 5%.

Fig. 3. Relationship between extracellular idarubicin concentration and DNA damage (rad equivalence). H-35 rat hepatoma cells in log growth were incubated with idarubicin at concentrations between 0.01 and 1.0 μM for 2 h. DNA damage was assessed by alkaline unwinding, with X-ray calibration. Data are presented as mean values ± standard errors for three to seven separate experiments.

Influence of Idarubicin on Cell Cycle Progression in the H-35 Rat Hepatoma. Bruce et al. first proposed the theory of cell-cycle phase specific cytotoxicity (33). The anthracycline antibiotics, as well as a variety of other antineoplastic drugs, produce a G2/M block in growing cells (34). Similarly, idarubicin produced a G2/M block in the H-35 rat hepatoma following a 2-h exposure to drug. Whereas control cells in log phase showed a distribution ratio of G1:S to G2:M of 62:38, after 2 h of treatment with 0.05 μM idarubicin, the ratio of cells in G1:S to G2:M was 37:63.

Fig. 4. Relationship between the extracellular idarubicin concentration and the induction of DNA damage or residual cell growth. Incubation conditions, alkaline unwinding and MTT assays were performed as described in Figs. 1 and 3 and the “Methods” section.

Effects of Idarubicin on Macromolecular Biosynthesis. The effects of idarubicin on the cellular synthesis of DNA, RNA, and protein by the H-35 rat hepatoma were assessed in order to explore the nature of the drug-induced lesions which may be associated with the inhibition of cell proliferation. Fig. 2 demonstrates that idarubicin produces a concentration-dependent inhibition of DNA synthesis with an IC50 value of approximately 0.5 μM, but demonstrates only a minimal effect on RNA synthesis and virtually no effect on protein synthesis.

Induction of DNA Cleavage. Fig. 3 demonstrates a direct correspondence between the extracellular idarubicin concentration and DNA cleavage (single-strand breaks) as assessed using the alkaline unwinding assay. (The straight line drawn through these points is based on a correlation coefficient of approximately 0.98). At a concentration of idarubicin where growth is inhibited by greater than 70% (0.05 μM), DNA damage is equivalent to approximately 150 rads of X-irradiation.

Fig. 5 presents the results of a representative alkaline elution assay designed to discriminate between direct and protein-associated DNA damage in the rat hepatoma cell exposed to 0.1 μM idarubicin. While cell lysis in the presence of proteinase K results in the expression of DNA damage in excess of that observed in the absence of enzymatic deproteinization, it is evident that idarubicin also induces DNA cleavage which is not protein concealed.

Neutral elution studies were performed to monitor double-strand breaks in DNA as Goldenberg et al. have reported a close correspondence between induction of double-strand

4 This IC50 value for daunorubicin is higher than reported previously (23). With continued passage in cell culture, modest differences in sensitivity of the hepatoma cell to certain antineoplastic drugs is observed.
and DNR were not statistically significant (P < 0.5).

and 32.1 ±5.2% of accumulated daunorubicin (as reported previously) (23). Analysis of these data using the Student's t-test showed that hepatoocytes retained 27.1 ± 4.3% of accumulated idarubicin, the hepatoocytes after a 2-h incubation. In experiments monitoring leakage of the cytoplasmic enzyme, glutamic oxaloacetate transaminase, as an index of gross damage to the plasma membranes following drug treatment. There appeared to be minimal leakage of GOT from the hepatoocytes following a 2-h treatment with IDR. In contrast, carbon tetrachloride, utilized as a positive control, clearly produced major changes in the plasma membranes.

Clinical Significance of Drug Accumulation and Retention. The data showed that cells differed in their ability to accumulate drugs even after normalization for protein content. This difference in drug accumulation has been observed in other studies (13, 14, 36, 37). Therefore, studies of drug accumulation are useful in predicting drug efficacy and resistance.

Influence of Free Radical Scavengers and the Iron Chelator, Deferoxamine, on Inhibition of Cell Growth by Idarubicin. The induction of drug resistance by idarubicin of direct or nonprotein associated DNA cleavage in the tumor cell line (13, 14, 36, 37) indicate that evaluation of the effect of free radical scavengers on drug-induced inhibition of H-35 hepatoma cell growth was required. The H-35 hepatoma cell was protected from the effects of 0.1 µM IDR by the free radical scavengers mannitol, catalase, methanol, or DMSO. While treatment with 0.1 µM IDR alone inhibited cell proliferation by 81.6 ± 4.6%, in the presence of 0.5 mM DMSO, proliferation was reduced by 83.0 ± 6.5%; in the presence of 50 mM mannitol, growth was reduced by 79.3 ± 8.4%; with 150 mM methanol, the reduction was 84.6 ± 4.1%; and with 1,500 units/ml catalase, 72.6 ± 6.4%.

The iron chelator, deferoxamine, which would limit iron-catalyzed free radical production, also failed to protect against IDR toxicity. In the presence of 0.5 mM deferoxamine and 0.1 µM IDR, proliferation was reduced by 80.7 ± 7.6%; in this series of experiments, a reduction of 78.8 ± 6.7% was observed using idarubicin alone.

Intracellular Accumulation and Retention of Drug. Cellular resistance to the anthracycline antibiotics has been shown to be related to alterations in membrane transport (i.e., reduced accumulation as a function of enhanced efflux) in a number of cell lines (38). Consequently, the enhanced toxicity and/or increased DNA damage produced by idarubicin in the H-35 hepatoma cell as compared to daunorubicin (23) could be a function of differential drug accumulation and/or retention. However, as Fig. 6 demonstrates, there were no apparent differences in cellular levels of DNR and IDR in the H-35 rat hepatoma after a 2-h incubation. In experiments monitoring retention of daunorubicin and idarubicin (at 0.5 µM), the hepatoma cells retained 27.1 ± 4.3% of accumulated idarubicin, and 32.1 ± 5.2% of accumulated daunorubicin (as reported previously) (23). Analysis of these data using the Student's t-test indicated that differences in retention of IDR and DNR were not statistically significant (P < 0.5).

**DISCUSSION**

Studies by Dehove et al. (21) and Rowley et al. (22) have demonstrated that the H-35 hepatoma cell is relatively insensitive to the anthracycline antibiotics in vitro as well as in a rat model. This tumor cell line can be considered “intrinsically resistant” to these drugs and may serve as a useful model for studying the mechanisms of drug resistance. However, the results of this study suggest that the anthracyclines may be more effective in vivo than previously thought.

**Table 1 Influence of IDR on membrane integrity**

<table>
<thead>
<tr>
<th>GOT levels in medium</th>
<th>% Cells stained</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.019 ± 0.007</td>
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<tr>
<td>0.05 µM IDR</td>
<td>0.033 ± 0.010</td>
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<tr>
<td>0.1 µM IDR</td>
<td>0.033 ± 0.034</td>
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<tr>
<td>0.5 µM IDR</td>
<td>0.038 ± 0.017</td>
</tr>
<tr>
<td>1.0 µM IDR</td>
<td>0.031 ± 0.010</td>
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<tr>
<td>5.0 mM CC14</td>
<td>0.114 ± 0.026</td>
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</table>

**Fig. 6.** Daunorubicin and idarubicin levels in H-35 rat hepatoma. H-35 rat hepatoma cells in log growth were incubated with either daunorubicin or idarubicin at concentrations between 0.05 and 1.0 µM. Results were normalized for determination of pmol/µg cell protein. Data are presented as mean values ± standard error for three to six individual experiments.
associated damage, which is thought to be associated with P388 leukemia (35, 46).

However, it is difficult to state conclusively that the similar problem is evident in studies of anthracycline interaction.

Inhibition of cell proliferation by a factor of approximately 20.

A number is associated with small increases in DNA cleavage. A IC50 value for inhibition of DNA synthesis exceeds that for the IC50 value for... (41-43). The relative ineffectiveness of the anthracyclines in clusters at approximately 30 nM cancer cells, HL-60 leukemia and Sarcoma 180, the IC50 for the anthracycline antibiotics clusters at approximately 30 nM (41-43). The relative ineffectiveness of the anthracyclines in inhibiting growth of the hepatoma cell may prove to be related to the origin of this hepatic tumor by treatment of rats with the hepatocarcinogen, aminoacetylfluorene (44). Carr and Laishes have reported that transformation of hepatocellular lesions by a carcinogenic stimulus selects for cells resistant to carcinogens and cross-resistant to antineoplastic drugs such as the anthracycline antibiotics (45).

The H-35 rat hepatoma is markedly more sensitive to the daunorubicin analogue, idarubicin, than to the parent compound or to Adriamycin. The relative sensitivity of the rat hepatoma cell to idarubicin as compared with daunorubicin (in terms of growth inhibition) parallels that reported by Capranico et al. in P388 leukemia cells (46). However, the basis for the enhanced sensitivity of the hepatoma cell to idarubicin as compared to daunorubicin is not as clearly evident as in the studies by Capranico et al. These authors report that idarubicin levels in the P388 leukemia cell exceed those for daunorubicin by 15-fold (46). In contrast, essentially identical levels of daunorubicin and its 4-demethoxy analogue are observed in the rat hepatoma cell. Furthermore, both drugs are retained to the same extent after a 2-h incubation in drug-free medium.

Consistent with reports in other tumor cell lines (47), incubation with idarubicin for 2 h produces a concentration-dependent inhibition of DNA synthesis, with minimal inhibition of RNA synthesis and virtually no effect on protein synthesis. However, inhibition of DNA synthesis does not appear to correspond with the inhibition of cellular proliferation, as the IC50 value for inhibition of DNA synthesis exceeds that for the inhibition of cell proliferation by a factor of approximately 20.

The present studies appear to suggest that the enhanced sensitivity of the rat hepatoma cell to idarubicin is a consequence of its capacity to induce DNA damage (single strand cleavage). However, it is difficult to state conclusively that the antiproliferative activity of IDR in the hepatoma cell is related to this DNA lesion since an extremely steep decline in cell number is associated with small increases in DNA cleavage. A similar problem is evident in studies of anthracycline interaction with P388 leukemia (35, 46).

DNA cleavage induced by idarubicin in the hepatoma cell appears to be composed of both direct damage, and protein-associated damage, which is thought to be associated with inhibition of Topoisomerase II (48). The report by Capranico et al. also demonstrated direct DNA damage produced by IDR (Fig. 4), although this point was overlooked in the text (46). The induction of nonprotein-associated DNA damage would appear to suggest involvement of free radicals in idarubicin toxicity. Potmesil et al. demonstrated that free radical scavengers protected L1210 murine leukemic cells against Adriamycin cytotoxicity as well as the direct DNA damage (nonprotein-associated single strand breaks) induced by high concentrations of Adriamycin (49). The lack of protection from idarubicin toxicity by free radical scavengers and deferoxamine and the absence of apparent damage to cellular membrane or mitochondrial integrity in the present studies suggest that free radical mechanisms do not contribute to idarubicin toxicity in the rat hepatoma cell. In this context, studies have indicated that the hepatoma cell appears to have high levels of endogenous glutathione peroxidase, glutathione transferase, and glutathione reductase, enzyme activities which would tend to counteract free-radical associated damage. It is also possible that low levels of cytochrome P450 reductase in the H-35 rat hepatoma (as indicated by the lack of metabolic conversion of the anthraclines to deoxyglucosine derivatives) (23) limits activation of anthracycles to free radical species in this tumor cell line.

While the induction of both direct and protein-associated DNA strand breaks may be responsible for the antiproliferative effects of idarubicin in the hepatoma cell, the absence of DNA double strand breaks in hepatoma cells exposed to idarubicin is unexpected, as Goldenberg et al. reported that DNA double-strand breaks appear to be associated with toxicity of the anthracycles in P388 leukemia cells (35). In view of the lack of close correlation between induction of DNA cleavage and antiproliferative activity of idarubicin, an alternative possibility which should be considered is that a minimal number of lesions at "hypersensitive" sites on DNA may mediate the antiproliferative effects of this anthracycline analogue in the hepatoma cell.

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REFERENCES


Table 2 Percentage decrease in mitochondrial succinate dehydrogenase activity

<table>
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<tr>
<th>[IDR]</th>
<th>% Decrease in succinate dehydrogenase activity (relative to control)</th>
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<tbody>
<tr>
<td>0.05 μM</td>
<td>11.7 ± 5.2</td>
</tr>
<tr>
<td>0.1 μM</td>
<td>12.3 ± 7.5</td>
</tr>
<tr>
<td>0.5 μM</td>
<td>8.0 ± 4.9</td>
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<tr>
<td>1.0 μM</td>
<td>8.7 ± 8.7</td>
</tr>
<tr>
<td>Cyanide (1 mM)</td>
<td>67.7 ± 14.4</td>
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</table>

Table 2 Percentage decrease in mitochondrial succinate dehydrogenase activity

Succinate dehydrogenase activity in the rat hepatoma cell was monitored following 2 h of incubation with the indicated drugs using the MTT tetrazolium dye assay as described previously. Data are presented ± standard error for four separate experiments.


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