Relationship of Adriamycin Concentrations to the DNA Lesions Induced in Hypoxic and Euxic L1210 Cells

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ABSTRACT

Exponentially growing L1210 mouse leukemia cells were incubated with Adriamycin (ADR) under hypoxic (95% N2:5% CO2) or euxic conditions (95% air:5% CO2) for 1 hr at 37° at a drug concentration ranging from 2.8 x 10^{-8} to 2.8 x 10^{-4} M, i.e., from levels attained clinically by bolus delivery to the high levels used in a i.p. drug dwell or experimentally, in vitro conditions. High-pressure liquid chromatography analyses showed diminishing efficiency in drug uptake by the cells as the dose was increased. There were no significant differences between hypoxic and euxic cells in drug uptake and metabolism. The frequency of DNA protein-associated single-strand breaks and DNA-protein cross-links per 10^6 nucleotides, detected by the alkaline elution technique, increased with the dose in the range of 2.8 x 10^{-8} to 2.8 x 10^{-4} M in both euxic and hypoxic cells and declined thereafter. However, the number of DNA lesions relative to a normalized drug level declined steadily, starting with the 2.8 x 10^{-5} M concentration. Concentrations >2.8 x 10^{-4} M of ADR induced still another type of lesion, direct DNA strand breaks, only in euxic cells. The results indicate that a common mechanism of interaction between drug and DNA is present in hypoxic and euxic cells at low ADR, while an O2-dependent mechanism becomes operational in euxic cells at high ADR levels.

INTRODUCTION

The 2 prototype anthracycline anticancer agents, ADR3 and daunorubicin, exert a variety of effects on cellular components (24, 25, 42) and cell metabolism and function (6, 11, 39). Among these effects, the inhibition of DNA replication and RNA transcription is considered template related and a consequence of DNA degradation (11, 44). Cytotoxicity may result from direct or mediated interaction between the drug and DNA. In addition to damage following drug binding to DNA (27), DNA break production by anthracycline-mediated free radicals (1–3) has been suggested as a mechanism of drug cytotoxicity. In the former case, the distortion of the DNA helix induced by intercalators such as ADR may cause the activation of endonucleases, possibly of the distortion of the DNA helix induced by intercalators such as ADR acting as a bioreductive alkylating agent under hypoxic conditions (41). Furthermore, some anthracyclines such as AD32 and its metabolites do not bind to DNA (15, 38) and still cause inhibition of DNA and RNA synthesis (8) and DNA strand breaks and cross-links (22) similar to those produced by ADR.

Although DNA-protein-associated SSB and DNA-protein cross-links by themselves may not be cytotoxic (34, 36), their frequency appears to increase with ADR concentration and ADR-induced cell kill, at least in the 10^{-7} to 10^{-5} M range (33–36). The lesions seem to be implicated in repair of DNA damaged by the intercalating agent ADR or by DNA nonbinding AD32 (30–32). In order to further elucidate the role of DNA protein-associated SSB and DNA-protein cross-links in ADR: DNA interactions, we have determined the effects of ADR concentration on the induction of DNA lesions in hypoxic and euxic cells. The wide range of concentrations chosen spans over 5 logs and is relevant to clinical situations and experimental models. The rate of DNA lesion production was correlated with drug uptake and metabolism. Some of the data published in this article have been reported in a preliminary form (29).

MATERIALS AND METHODS

Propagation and Radioactive Labeling of Tumor Cells. L1210 leukemia mouse cells (obtained from the Memorial Sloan-Kettering Cancer Center, Walker Laboratories, Rye, N. Y.) were grown in a suspension culture in McCoy’s Medium SA (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% heat-inactivated (56° for 30 min) fetal calf serum (Biofluids, Inc., Rockville, Md.) and 1% Pen-Strep (Grand Island Biological Co.). Exponentially growing cells with an 18-hr doubling time were labeled for 21 hr with either [methyl-3H]thymidine (40 to 60 mCi/ mmol; 0.1 µCi/ml) or [2-14C]thymidine (>50 mCi/mmol; 0.025 µCi/ml). Both isotopes were obtained from Amersham/Searle Corp., Arlington Heights, Ill. The labeling was terminated by incubation in nonradioactive medium at 4°.

Drug Treatment under Euxic or Hypoxic Conditions. ADR (NSC 123127; Farmitalia, Milan, Italy), diluted with sterile 0.15 M NaCl, was protected from light and stored at —85°. Ten ml of a cell suspension (10^6 cells/ml) in McCoy’s medium with 1% heat inactivated serum were loaded into each Wheaton Celstir suspension culture flask (Wheaton Scientific, Millville, N. J.) fitted with tubings impermeable to O2 (Nylo-Seal Tubing R8-NF; T.E. Conklin Co., New York, N. Y.) which provided a continuous flow (0.3 liter/min) of humidified 95% N2:5% CO2 or 95% air:5% CO2 (Union Carbide Corp., New York, N. Y.). The cell suspensions were exposed to the N2 or air atmosphere for 1 hr prior to drug treatment, with the drug solution being placed in a test tube inside each flask to allow equilibration with the gas mixture. The gassing continued for another hr during the drug exposure. The suspensions were continuously mixed with a magnetic stirrer at 37°. Except for the dose of 5.6 x 10^{-5} M (32.4 µg/ml), the concentrations ranged from 2.8 x 10^{-6} M (0.016 µg/ml) to 2.8 x 10^{-4} M (162.0 µg/ml) in log increments. In all instances, the nisms of DNA damage may also be operational; for example, ADR acting as a bioreductive alkylating agent under hypoxic conditions (41). Furthermore, some anthracyclines such as AD32 and its metabolites do not bind to DNA (15, 38) and still cause inhibition of DNA and RNA synthesis (8) and DNA strand breaks and cross-links (22) similar to those produced by ADR.

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2 To whom requests for reprints should be addressed, at Department of Radiology, New York University School of Medicine, 550 First Avenue, New York, N. Y. 10016.
3 The abbreviations used are: ADR, Adriamycin (doxorubicin); AD32, N-fluorooxycarbonyladriamycin-14-valerate; SSB, single-strand breaks; HPLC, high-pressure liquid chromatography; AMNOL, adriamycinol; SF, surviving fraction of clonogenic cells assessed by soft-agar technique.

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drug was added in 10- or 100-μl volumes per 1 ml of a cell suspension. Corresponding volumes of 0.15 M NaCl were added to control untreated samples. At the end of incubation, cells were washed twice with medium before further processing.

The experimental conditions of a 2-hr euoxia or hypoxia did not appreciably change the pH or glucose content of incubated media or the trypan blue exclusion (≥98%) or clonogenicity (plating efficiency, ~60%) of exposed cells. The procedure led to hypoxia with an oxygen enhancement ratio of approximately 2.9. The experimental procedure for induction of hypoxia and euoxia is similar to the one used by others for the testing of radiosensitizing compounds (23) and chemotherapeutic agents (40).

HPLC Analysis. Cells incubated with various concentrations of ADR for 1 hr and control untreated cells were washed 2 times in phosphate-buffered saline (0.15 M NaCl, 0.71 mM KH₂PO₄, and 4.28 mM K₂HPO₄, pH 7.4) and sonicated at 0°C. Both the cell sonicates and the incubation media were frozen in liquid N₂ and stored at −85°C and later melted and extracted with 2 × 3 ml of ethyl acetate:n-propanol alcohol (8:1, v/v). The extracts were evaporated to dryness under a stream of N₂ and reconstituted in 50 to 200 μl of methanol.

The concentrations of ADR, AMNOL, other metabolites, and aglycones were determined using reverse-phase HPLC. Aliquots (5 to 10 μl) of the methanol-reconstituted extracts were injected for analysis. ADR and metabolites were identified by their retention times relative to known standards and were quantified by their respective standard curves and recovery of known compounds. Unidentified metabolites were characterized according to their retention times.

The HPLC methodology used in this study, including determination of the extraction efficiency of ADR and AMNOL, is identical with the conditions described previously (17), but the quantification is improved by the use of an internal standard (16).

DNA Alkaline Elution. Details on this method have been described in several publications (18, 19, 22). "Experimental" cells (cells treated with the drug) were labeled with [3H]thymidine and assayed either directly or after exposure to 300 R of X-irradiation (X-ray unit, 220 kV; 26 ma, 216 R/mm) at 0°C. "Internal reference" [3H]thymidine-labeled cells were exposed only to 150 R at 0°C. The trypan blue exclusion of drug-treated and untreated control cells was better than 95%. A mixture of experimental and internal reference cells (6.5 × 10⁶ cells total) was deposited on polyvinyl chloride filters (Metrical membrane filter DM-800; Gelman Sciences, Inc., Ann Arbor, Mich.) at 2 to 4°C, washed with ice-cold phosphate-buffered saline, and lysed at room temperature over a 10-min interval with 0.2% Sarkosyl (ICN Pharmaceuticals, Inc., Cincinnati, Ohio and K and K Laboratories, Inc., Plainview, N. Y.).

Subsequently, the filters were incubated at room temperature for 50 min in the lysing solution with or without proteinase K (0.5 mg/ml; MCB Reagents, Norwood, Ohio). The filters were washed with 0.02 M trisodium EDTA, pH 10, and cell lysates eluted at room temperature in the dark at a flow of 0.035 ml/min. The eluting solution consisted of 0.02 M EDTA and tetrapropylammonium hydroxide (RSA Corp., Ardsley, N. Y.) added to adjust pH to 12.1. In tests for alkaline-labile sites (19), the pH was 12.6. Eluted fractions were collected at 90- or 120-min intervals over 12 to 16 hr. The radioactivity of individual fractions, as well as the radioactivity remaining on the filter at the end of the assay, was determined in a liquid scintillation counter.

Data were analyzed as described previously (19, 22). The results were expressed as frequencies of DNA direct SSB, DNA-protein-associated SSB, or DNA-protein cross-links per 10⁶ nucleotides.

**RESULTS**

Uptake and Metabolism of ADR. Chart 1 shows the intracellular uptake of ADR by L1210 cells. The cells were incubated in 2.8 × 10⁻⁸ to 2.8 × 10⁻⁵ M concentration in culture media under hypoxic or euoxic conditions. The cellular drug content increased gradually with the increasing dose (Chart 1A). When ADR fluorescence in cells, however, is expressed as relative to total ADR fluorescence (fluorescence in the media plus that in the cells), there is a 7- to 8-fold difference between the lowest and the highest dose (Chart 1B). Of the total fluorescence, 25% under hypoxic and 21.8% under euoxic conditions can be accounted for by the fluorescence in cells incubated with a 2.8 × 10⁻⁶ M concentration of ADR. This was significantly higher than 3.4 and 2.9% in hypoxic and 3.4 and 2.8% in euoxic cells treated with 2.8 × 10⁻⁵ M ADR. The fluorescent signals of ADR metabolites, detected at different retention times by reverse-phase HPLC and expressed as μg of ADR equivalents, are listed in Table 1. These signals do not correspond to AMNOL, 7-deoxydihydrodramycinone, 7-deoxy-13-dihydrodramycinone, or other known ADR metabolites. At comparable concentrations, there were no significant differences between hypoxic and euoxic conditions with respect to drug uptake and metabolism, except for a marginally significant difference in normalized uptake, shown in the legend of Chart 1.

Effect of ADR Concentration and Oxygehination on DNA Lesions. Table 2 summarizes the data obtained by alkaline elution. In drug-treated cells, the frequency of all DNA SSB (protein-associated plus direct) increased with the dose in the range of 2.8 × 10⁻⁶ to 2.8 × 10⁻⁵ M and reached a plateau in euoxic or declined in hypoxic cells. There is no statistically significant difference between the frequency of lesions induced in cells under hypoxia or euoxia except for the lowest and the highest drug concentration. Chart 2 shows the data points for DNA protein-associated SSB and direct SSB separately. It becomes apparent that the maximal frequency of protein-associated SSB is present again at 2.8 × 10⁻⁵ M concentration, and this is followed by a decrease. A similar dose response was...
Table 1
Concentration of ADR metabolites in cells and media

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Metabolite retention time (min)</th>
<th>Metabolite concentrations (µg ADR equivalent/ml) at following concentration of ADR (µ) in the incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.8 x 10^-7</td>
<td>2.8 x 10^-6</td>
</tr>
<tr>
<td></td>
<td>Cells</td>
<td>Media</td>
</tr>
<tr>
<td>Euoxic</td>
<td>1.59</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>2.99</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>4.04</td>
<td>ND</td>
</tr>
<tr>
<td>Hypoxic</td>
<td>1.59</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>2.99</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>4.04</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Reverse-phase retention times for ADR metabolites (min). Retention time for ADR is 2.30 min; ADR uptake, expressed as µg/10^6 cells, is shown in Chart 1.

Incubation time, 1 hr at 37°C.

ND, not detected.

Table 2
Overall incidence of DNA SSB in euoxic and hypoxic cells

The difference between SSB in euoxic and hypoxic cells is significant at 2.8 x 10^-6 M and 2.8 x 10^-5 M (p < 0.01, d.f. 3 to 4); the rest is insignificant (p > 0.6 to 0.1, d.f. 3 to 6). Differences between SSB induced in euoxic cells by 2.8 x 10^-5 M to 2.8 x 10^-4 M were not significant (p > 0.1, d.f. 9 to 10) and in hypoxic cells were highly significant (p < 0.001, d.f. 7 to 10).

<table>
<thead>
<tr>
<th>Concentration of ADR (M)</th>
<th>SSB/10^8 nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Euoxia</td>
</tr>
<tr>
<td>2.8 x 10^-7</td>
<td>-0.32 ± 0.30</td>
</tr>
<tr>
<td>2.8 x 10^-6</td>
<td>0.66 ± 0.16</td>
</tr>
<tr>
<td>2.8 x 10^-5</td>
<td>1.17 ± 0.56</td>
</tr>
<tr>
<td>2.8 x 10^-4</td>
<td>2.48 ± 0.63</td>
</tr>
<tr>
<td>5.6 x 10^-5</td>
<td>3.38 ± 1.21</td>
</tr>
<tr>
<td>2.8 x 10^-4</td>
<td>2.99 ± 1.24</td>
</tr>
<tr>
<td></td>
<td>Hypoxia</td>
</tr>
<tr>
<td>2.8 x 10^-7</td>
<td>0.22 ± 0.10</td>
</tr>
<tr>
<td>2.8 x 10^-6</td>
<td>0.96 ± 0.68</td>
</tr>
<tr>
<td>2.8 x 10^-5</td>
<td>2.73 ± 1.02</td>
</tr>
<tr>
<td>2.8 x 10^-4</td>
<td>4.32 ± 1.35</td>
</tr>
<tr>
<td>5.6 x 10^-5</td>
<td>2.39 ± 1.25</td>
</tr>
<tr>
<td>2.8 x 10^-4</td>
<td>1.23 ± 0.40</td>
</tr>
</tbody>
</table>

a Mean ± S.D. of all (direct plus protein-associated) SSB. Frequencies of both types of SSB separately are shown in Chart 2.

Chart 3. Efficiency of DNA lesion production in cells treated with various concentrations of ADR under euoxic (A, O) or hypoxic (A) conditions. A, incidence of DNA SSB (direct or protein-associated). C, direct SSB (air); A, protein-associated SSB (air); A, protein-associated SSB (N2). B, incidence of DNA-protein cross-links. A, DNA-protein cross-links (air); A, DNA-protein cross-links (N2). In both instances, the frequency of DNA lesions per 10^6 nucleotides is normalized to the 1.7 x 10^-6 M (1.0 µg/ml) concentration of ADR in incubation media. For the calculation of normalized frequencies of DNA breaks, the means of absolute numbers shown in Chart 2 have been used. Similar data for DNA-protein cross-links are not shown.

Correlation coefficient of the slopes, -0.9986 to -0.9726; n = 4 to 6.
associated SSB, with a significant difference between the 2 slopes ($p < 0.05$, d.f. 8).

Results similar to those obtained for the protein-associated SSB formation were also established for the relationship between ADR doses and the efficiency of DNA-protein cross-link production. The efficiency declines with the increasing concentration of the drug (Chart 3B) and shows no statistically significant difference between hypoxia and euoxia. Chart 4 presents several typical elution curves of DNA isolated from cells treated with various concentrations of ADR under euoxic and hypoxic conditions. The 3 highest concentrations ($2.8 \times 10^{-5}$ to $2.8 \times 10^{-4}$ M) cause an accelerated elution of DNA from lysates not treated with proteinase K and obtained from euoxic cells.

Tests for alkali-labile sites were performed using lysates of cells treated with $2.8 \times 10^{-4}$ M concentration of the drug under euoxic conditions. As shown in Table 3 that the elution rates of DNA obtained from control untreated cells or cells irradiated with 300 R are not affected by pH in the range of 12.1 to 12.6. The elution for cells treated with ADR, however, shows about a 40% increase in direct SSB at pH 12.6 over pH 12.1, and this indicates partial pH dependence of their formation (19).

In separate experiments, we have tested the possibility that ADR is released from drug-treated cells and could induce DNA breaks during preparatory steps and lysis preceding the elution. Aliquots of $3 \times 10^5$ $^{3}H$-labeled (no drug), $3 \times 10^5$ unlabelled (treated with $2.8 \times 10^{-4}$ M ADR) and $4 \times 10^5$ internal reference $^{3}H$-labeled cells were mixed before lysis, deposited on a filter, and processed as described in "Materials and Methods." $^{3}H$-labeled cells were irradiated with 150 R and unlabelled cells with 300 R. As already reported previously (22), these experiments did not show any DNA breaks caused by residual ADR present in cell lysates. Also, elution rates of internal reference $^{3}H$-labeled lysates were consistently comparable in mixtures with untreated controls and in mixtures with lysates of ADR-treated cells. This by itself is a strong indication that no significant number of DNA breaks is induced during the preparatory phase of alkaline elution.

### DISCUSSION

**DNA protein-associated SSB and DNA-protein cross-links**

**Chart 4.** Effects of various concentrations of ADR (m) on alkaline elution of DNA. Cells incubated with the drug (G) under euoxic or hypoxic conditions were unirradiated, and their lysates were without proteinase K treatment. Hatched areas, range of elution rate variation of untreated control cells (17 experiments). The mean ± S.D. of repeated experiments done with each drug concentration is shown in Chart 1.

High concentrations of ADR induce another type of lesion, DNA direct ("nonenzymatic") SSB, only in euoxic cells. Unlike protein-associated SSB, the dose response for the induction of direct SSB showed a linear increase. Some of the direct SSB have been detected in tumor cells treated in vitro with ADR (34-36) and with its DNA nonbinding analogue AD32 (22), with either of the 2 agents in vivo (30-32), and with a variety of other intercalators in vitro (18, 33, 34, 36, 45). The present investigation addresses 2 aspects of their formation, namely, dependency on ADR concentration and relationship to oxygenation. With the exception of the highest concentration, there was only a low level of ADR metabolites detected in cells and media. This indicates that ADR remains primarily unchanged and implicated in the induction of DNA changes. The response of DNA SSB to drug concentration has a peak at $2.8 \times 10^{-4}$ M in euoxic as well as in hypoxic cells. This is followed in hypoxic cells by a decrease in DNA breaks while, in euoxic cells, the plateau at high drug levels is due to the increasing incidence of direct SSB. In any case, protein-associated SSB show a paradoxical decrease starting at $2.8 \times 10^{-4}$ M exposure. A similar phenomenon has been noted with cell exposure to graded single doses of 1-β-arabinofuranosylcytosine (13) or of bisantrene (7). The mechanism responsible for this biphasic dose-dependent pattern may result from the incorporation of 1-β-arabinofuranosylcytosine in the DNA strand during replicative synthesis but remained unclear for bisantrene. When the incidence of protein-associated SSB in our experiments is expressed as relative to a fixed concentration of ADR (Chart 3), the number of DNA lesions tends to reach saturation over increasing concentrations. In other words, the efficiency of DNA lesion production decreases. Since the design of experiments with bisantrene was similar to ours, we have recalculated the data on frequencies of protein-associated SSB and protein-DNA cross-links (7) and have shown a trend which is identical to ours in the efficiency of DNA lesion production. While the mechanism responsible for this phenomenon remains unclear, several factors could be suggested (for example, a relative decrease of drug uptake by the cells with increasing drug concentration in media (Chart 1B; Ref. 7), an increase in binding to other macromolecules than DNA, a decreasing availability of substrates essential for enzymatic reactions resulting in DNA lesions, or a decrease in accessible regions for drug interaction with DNA).

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Direct SSB/10^6 nucleotides</th>
<th>Protein-associated SSB/10^6 nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.1</td>
<td>0.108</td>
<td>-0.068</td>
<td></td>
</tr>
<tr>
<td>12.6</td>
<td>0.058</td>
<td>-0.048</td>
<td></td>
</tr>
<tr>
<td>300 R</td>
<td>2.750</td>
<td>-0.150</td>
<td></td>
</tr>
<tr>
<td>12.6</td>
<td>2.801</td>
<td>-0.150</td>
<td></td>
</tr>
<tr>
<td>ADR, 2.8 x 10^{-4} M</td>
<td>1.771 ± 0.386^2</td>
<td>0.814 ± 0.120</td>
<td></td>
</tr>
<tr>
<td>ADR, 2.8 x 10^{-5} M</td>
<td>2.504 ± 0.443</td>
<td>0.752 ± 0.198</td>
<td></td>
</tr>
</tbody>
</table>

^2 These values should be compared with the "background" for DNA direct SSB in untreated cells (0.268 ± 0.285) and for protein-associated SSB (0.123 to 0.320). For more details, see "Materials and Methods" and the legend to Chart 2.

^3 Treated or untreated L1210 cells were eluted at pH 12.1 or 12.6 using the procedure described in "Materials and Methods."

^4 Mean ± S.D. of DNA direct SSB in cells irradiated with 300 R, 2.782 ± 0.354 (total of 14 experiments); protein-associated SSB are in the range of "background" values.

^5 Mean ± S.D. of 3 to 4 experiments. t-test: significant difference between direct SSB caused by ADR at elution pH 12.1 and 12.6 ($p < 0.01$, d.f. 5). Difference between protein-associated SSB not significant ($p > 0.1$, d.f. 6).
are alkali-labile sites converted to breaks in alkali conditions. Among the O₂-related effects leading to the production of direct SSB, a free radical mechanism should be considered.

The relevance of the above-mentioned findings has to be evaluated in a wider context of clinical and experimental studies. Should ADR be delivered as an i.v. bolus to a patient, peak plasma levels following a dose of 22.5 to 90 mg/sq m are 10⁻⁷ to 10⁻⁷ M concentration of the drug at 37°in air results in a ~10% survival of the clonogens, and concentrations >5 x 10⁻⁴ M. These include the induction of cytofluorescence in whole cells or in cell nuclei (12), detection of free-radical species in a reaction mixture containing the drug (1), O₂-mediated DNA cleavage produced by ADR semiquinone-free radicals (4), and the mode of generation of free radicals in a cell-free system or in a system with intact tumor cells in the presence of ADR (14, 37).

It has been shown repeatedly that a 1-hr incubation with a 10⁻⁷ to 10⁻⁴ M concentration of the drug at 37° in air results in an ~10% survival of the clonogens, and concentrations >5 x 10⁻⁴ M decrease the SF to >10⁻⁴ (9, 36, 41, 43, 45). This can be compared with a SF of 0.35 ± 0.05 after an exposure to 0.87 x 10⁻⁷ M of ADR and a SF of 0.05 ± 0.01 after a 1.8 x 10⁻⁶ M exposure, both being dose-responses of the L1210 line used in our experiments.⁸ The drug concentrations ranging from sub-toxic to highly toxic were instrumental in showing DNA lesions which otherwise, should a narrower dose range be used, would remain undetected. This experimental design also helped to establish the relationship between drug concentration in media, drug partitioning into the cellular compartment, and efficiency of DNA lesion formation.

Since the nature of DNA lesions induced by ADR and measured by alkaline elution is not fully understood, the simple comparison of various concentrations producing different amounts of DNA protein-associated or direct SSB is only an initial step in exploring the mechanism(s) underlying their production. Ongoing studies indicate that DNA direct SSB caused in cells by high ADR concentrations can be at least partially prevented by antioxidant agents (28). It is hoped that a more enlightened understanding of ADR/DNA interactions may help in developing new anticyclic agents.

REFERENCES


* M. Levin, unpublished observations.


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