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PROTEIN-ASSOCIATED DEOXYRIBONUCLEIC ACID STRAND BREAKS PRODUCED IN MOUSE LEUKEMIA L1210 CELLS BY ELLIPTICINE AND 2-METHYL-9-HYDROXYELLIPTICINIUM

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Abstract—DNA intercalating agents, including ellipticine, had been found previously to produce protein-associated DNA single-strand breaks and double-strand breaks in mammalian cells. The relationship between these effects on DNA and cytotoxicity could not be determined reliably for ellipticine, because of the poor solubility characteristics of this compound. Studies were therefore carried out using the cationic derivative, 2-methyl-9-hydroxyellipticinium (2-Me-9-OH-E$^+$), which has adequate water solubility and retains antitumor activity. DNA single-strand breaks (SSB) and DNA–protein crosslinks (DPC) were measured using the alkaline elution (pH 12) technique, and double-strand breaks (DSB) were measured by the neutral elution (pH 10) method. The effects of ellipticine and 2-Me-9-OH-E$^+$ were compared in mouse leukemia L1210 cells. Like ellipticine, moderate concentrations of 2-Me-9-OH-E$^+$ produced protein-associated SSB, indicated by the appearance of SSB and DPC at approximately equal frequencies and localized with respect to each other. Below 20 μM (1-hr treatments), the effects of the two drugs were comparable in magnitude. At higher concentrations, ellipticine produced extensive DNA breakage not associated with protein; this is perhaps secondary to an action on membranes or other non-DNA targets. However, 2-Me-9-OH-E$^+$ produced only protein-associated strand breaks, even at 4-fold higher concentrations. The two compounds produced similar and relatively large extents of double-strand scission. The measured DSB/SSB ratio was higher than that produced by X-ray or certain other intercalators that have been similarly studied. The DNA effects of 2-Me-9-OH-E$^+$, unlike those of ellipticine, were readily reversible and, therefore, permitted a meaningful correspondence between the magnitudes of the DNA effects and the inhibition of colony-forming ability. Comparison with two other types of intercalating agents indicated that neither the SSB nor the DSB predicts the magnitude of cell killing.

Intercalating agents produce breaks in the DNA of treated cells [1–6]. These breaks differ from those produced by other DNA reactive agents or by ionizing radiation in that they are associated with protein apparently covalently bound to DNA at or near the break site [6]. Whether these breaks are mechanistically related to the cytotoxic effects of intercalators is unknown [5, 6].

Although almost all intercalating agents examined produce protein-associated DNA breaks, the agents may differ in the rates of formation and resealing of the breaks, in the ratio of single-strand breaks (SSB) to double-strand breaks (DSB), and in cytotoxicity relative to a given frequency of strand breaks [2, 5–7]. To determine the magnitude of the cytotoxicity which corresponds to a given frequency of intercalator-induced strand breaks, one must be able to remove the drug from the cells at the end of the treatment period, so that surviving cells can then proliferate to form colonies. We have previously studied the actions of representatives of three chemical classes of intercalators in L1210 cells. The 9-aminoacridine derivative, 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide (m-AMSA)†, was readily removed by washing the cells, and the protein-associated strand breaks induced by this compound were largely reversed within 15 min [6]. In the case of the anthracycline, adriamycin, however, much of the drug was retained despite cell washing, and most of the protein-associated strand breakage persisted for many hours [6]. Adriamycin showed a relatively high cytotoxicity, relative to the frequency of protein-associated strand breaks, but this must have been at least partly due to persistence of drug in washed cells [6]. The anthracycline, 5-iminodaunorubicin, on the other hand, produced readily reversible effects in the system, so that cytotoxicity and DNA effects could be meaningfully compared [7].

The current work deals with a third class of intercalators, the ellipticines. Ellipticine itself has been shown previously to produce protein-associated DNA breaks, but the effect was not easily reversed

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† Abbreviations: m-AMSA, 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide; 2-Me-9-OH-E$^+$, 2-methyl-9-hydroxyellipticinium cation; RPMI 1630, Roswell Park Memorial Institute medium 1630; and SDS, sodium dodecylsulfate.

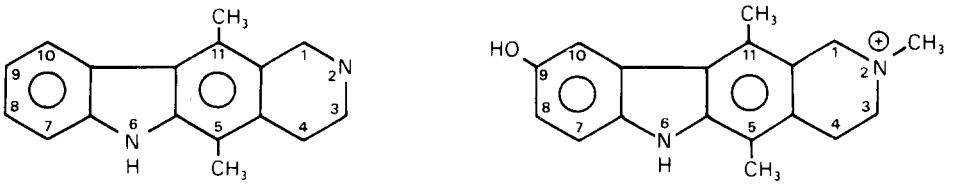

Fig. 1. Structures of ellipticine and 2-methyl-9-hydroxyellipticinium cation (2-Me-9-OH-E$^+$).

[3, 4, 8]. It may be difficult to remove ellipticine from cells because of its lipophilicity and tendency to adsorb to surfaces [9, 10]. Another difficulty is that the compound may produce cytotoxic effects due to actions on the cell surface or on cell membranes, as suggested by the tendency of ellipticine to cause hemolysis [11].

We have attempted to avoid these difficulties by studying the cationic ellipticine derivative, 2-methyl-9-hydroxyellipticinium (2-Me-9-OH-E$^+$) (Fig. 1), which has been reported to have substantial antitumor activity (Refs. 12 and 13; J. B. LePecq, personal communication). In solution this compound is always in the cationic state, whereas ellipticine is in acid-base equilibrium near physiologic pH. Hence, 2-Me-9-OH-E$^+$ is less lipophilic and more water soluble than ellipticine. Since ellipticine probably binds to DNA as the cation, it is likely that 2-Me-9-OH-E$^+$ binds to DNA in a similar manner. We report here that 2-Me-9-OH-E$^+$ produced rapidly reversible protein-associated DNA breaks and that the ratio of double-strand to single-strand breaks is similar to that produced by ellipticine and higher than that produced by other intercalators.

MATERIALS AND METHODS

Cells and radioactive labeling. Mouse leukemia L1210 cells were grown in suspension culture in RPMI 1630 medium, supplemented with 15% fetal calf serum plus penicillin and streptomycin. Stock cultures were maintained in static bottles without antibiotics and were used to initiate suspension cultures. Cultures utilized to assess drug effects were in exponential growth phase with a doubling time of 13–15 hr.

Cellular DNA was radioactively labeled in exponentially growing cells by incubation for 20 hr at 37° with [2-^{14}C]thymidine (0.01 μCi/ml) or with [methyl-^{3}H]thymidine (0.1 μCi/ml, 10^{-6} M unlabeled thymidine added), (New England Nuclear Corp., Boston, MA).

Drugs and drug treatment. 2-Methyl-9-hydroxyellipticinium acetate was the gift of Dr. J. B. LePecq, Laboratoire de Pharmacologie Moleculaire au CNRS, Institut Gustave-Roussy, Villejuif, France. Stock drug (8.25 mM) was dissolved in glass-distilled water and stored frozen. Ellipticine (NSC 71795) was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD. Stock drug (1 mM) was dissolved in 0.1 N HCl and refrigerated in plastic tubes. All drug treatments were for 1 hr at 37° unless otherwise indicated.

Cell survival. Cells were treated as above and assayed for colony-forming ability in soft agar by the method of Chu and Fisher [14].

Filter elution assays. The elution methodology has been described by Kohn [15], Kohn, *et al.* [16], Bradley and Kohn [17], Woods [18] and Zwelling *et al.* [6]. All drug treatments were as described above. Drug was removed by centrifugation at ice temperature with resuspension in ice-cold medium.

DNA–protein crosslinking. Drug-treated (or untreated control) ^{14}C-thymidine-labeled cells were X-irradiated at ice temperature with 3000 rads. These cells were combined with an equal number (approximately 5×10^5) of ^{3}H-thymidine-labeled cells which had received no drug treatment and had been concurrently irradiated, and the cells were deposited on 2 μm polyvinyl chloride filters (Type BS, Millipore, Bedford, MA) by gentle suction. Cell lysis was effected with 2 M NaCl, 0.2% Sarkosyl, 0.04 M EDTA, pH 10.0 (5 ml) and this solution was removed by washing the filter with 0.04 M EDTA, pH 10.0 (3 ml). DNA elution was performed with tetrapropylammonium hydroxide-EDTA, pH 12.1. Fractions (6 ml) were collected every 3 hr for 15 hr. Samples were processed and data were computed as previously described. DNA–protein crosslinks were calculated using the bound-to-one-terminus model of Ross *et al.* [4] as follows:

$$P_X = [(1 - r)^{-1} - (1 - r_0)^{-1}]P_B \qquad (1)$$

where P_B is the DNA single-strand break frequency produced by the X-irradiation (3000 rads). P_X is thus expressed in single-strand break (SSB) rad-equivalents (see Ref. 6). The symbols r_0 and r represent retention of DNA on filters from 3000 R-irradiated ^{3}H- and ^{14}C-thymidine-labeled cells respectively. The degree to which r exceeds r_0 is a measure of DNA–protein crosslinking (see Refs. 6 and 16).

DNA single-strand breaks. Cells, ^{14}C-thymidine-labeled as described above, either were drug treated or were untreated controls. Untreated ^{3}H-thymidine-labeled cells received 300 rads at ice temperature and served as internal standard cells (see Refs. 6 and 16). Again, approximately equal numbers of cells were mixed and deposited on filters, but for this assay filters were 2 μm polycarbonate (Nucleopore Corp., Pleasanton, CA). Lysis was effected with 5 ml of 2% sodium dodecylsulfate (SDS), 0.1 M glycine and 0.025 M disodium EDTA, pH 10. Elution was then begun with tetrapropylammonium hydroxide-EDTA–0.1% SDS, pH 12.1, layered above 2 ml of 0.5 mg/ml proteinase-K dissolved in the 2% SDS lysing solution. Sample collection

over 15 hr was as previously described at a pump speed of 0.03 to 0.04 ml/min. Single-strand break frequency (in SSB-rad-equivalents) was calculated by the formula:

$$\text{Break frequency} = \frac{\log (r_1/r_0)}{\log (R_0/r_0)} P_B \qquad (2)$$

where $P_B = 300$ rads, and r_1, r_0 and R_0 are relative retentions (see Ref. 6) of DNA from drug-treated, untreated and 300 rad-treated ^{14}C-labeled cells respectively. This retention is taken as the point during the elution when 35% of the [^{3}H]DNA remains on the filter (see Refs. 6 and 16).

A few experiments were performed using the low sensitivity alkaline elution assay as previously described (pump speed = 0.12 to 0.16 ml/min) [6]. This was used initially to evaluate drug concentrations producing high break frequencies. Equation 2 was still utilized to calculate break frequencies (see Refs. 6 and 16).

Double-strand breaks. DNA double-strand breaks were measured by a modification of the method of Bradley and Kohn [17]. Approximately 2.5×10^5 ^{14}C-thymidine-labeled cells were deposited on a polycarbonate filter and directly eluted with tetrapropylammonium hydroxide-EDTA–0.1% SDS, pH 9.6, layered above 2 ml of the proteinase-K solution described above. Otherwise, elution, sample collection and sample processing were identical to the single-strand break assay. The fraction of [^{14}C]DNA retained in each fraction was plotted versus time of elution. The retention of this DNA at 10 hr of elution is a measure of double-strand breaks used and was expressed in double-strand break (DSB) rad-equivalents by comparing the retention of drug-treated cells with a standard curve generated by assays performed using X-irradiated cells. This standard curve is the plot of the retention of DNA at 10 hr of elution (R) of X-irradiated cells versus the administered X-ray dose in kilorads (x) and has the formula:

$$R = -0.083x + 0.84 \, (r = -0.97) \qquad (3)$$

To compare the single- and double-strand breaks produced by various drugs, a derivation was needed which related these quantities to an agent in which the ratio of actual single-strand breaks (excluding those arising from double-strand breaks, i.e. two opposed single-strand breaks) versus actual double-strand breaks (k_{RS}/k_{RD}) was known. Literature values for X-radiation place this between 10 and 40 [19–22]. The formula for the ratio of actual single-strand breaks, s, to actual double-strand breaks, d, is given as:

$$\frac{s}{d} = \frac{k_{RS}}{k_{RB}} \frac{[\text{SSB}]}{[\text{DSB}]} - 2 \qquad (4)$$

where [SSB] and [DSB] are the measured break frequencies in rad-equivalents for the two assays. Thus, if a drug produced solely double-strand breaks, s would equal 0 and the measured values for [SSB]/[DSB] would fall between 0.05 and 0.2 for k_{RS}/k_{RD} equal to 40 and 10 respectively [19–22].

RESULTS

The abilities of ellipticine and 2-Me-9-OH-E$^+$ to reduce colony formation of L1210 cells is shown in Fig. 2. In the case of 2-Me-9-OH-E$^+$, the shape of the plot of log (survival fraction) versus concentration suggests the presence of a resistant component of the L1210 cell population, a saturable mechanism of 2-Me-9-OH-E$^+$ uptake, or depletion of a key intracellular co-factor. More importantly, at doses used in subsequent studies, ellipticine produced less killing than 2-Me-9-OH-E$^+$.

The frequency of DNA single-strand break production by these two agents is shown in Fig. 3. Increasing 2-Me-9-OH-E$^+$ concentrations produced increasing break frequencies, up to approximately 15–35 μM. At higher concentrations, a leveling tendency in break production became apparent which may have been caused by factors similar to those hypothesized to affect the shape of the 2-Me-9-OH-E$^+$ survival curve (Fig. 2) (*vide supra*). Ellipticine also produced increasing DNA breaks with increasing concentration, but valid results could only be obtained using 20 μM ellipticine or less because, at higher concentrations, single-strand breaks appeared that were not protein-associated (Fig. 4). Breaks not associated with protein are detected in elution assays which do not include deproteinization. No such breaks were detected in 66 μM 2-Me-9-OH-E$^+$-treated cells or in 20 μM ellipticine-treated cells. However, 40 and 60 μM ellipticine did produce DNA fragmentation detectable in assays without proteinase. These elution curves may represent the combined effects of DNA from dead or dying cells and protein-associated breaks, or they may actually

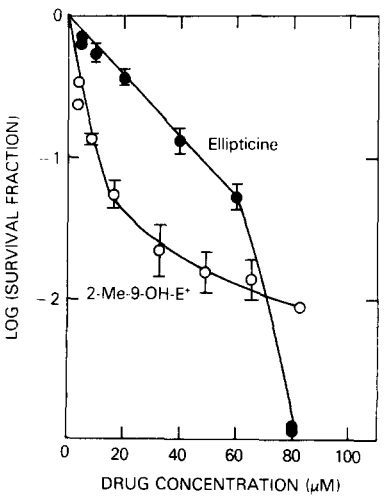

Fig. 2. Survival of mouse leukemia L1210 cells treated with various concentrations of ellipticine (●) or 2-methyl-9-hydroxyellipticinium (○). L1210 cells in exponential growth phase were treated for 60 min at 37°. Colony formation was quantified by soft-agar cloning [14]. Each point is the mean from at least three replicate tubes within an experiment. Error bars indicate ± 1 S.E.M. for three or more independent experiments. If less than three experiments, individual points are shown.

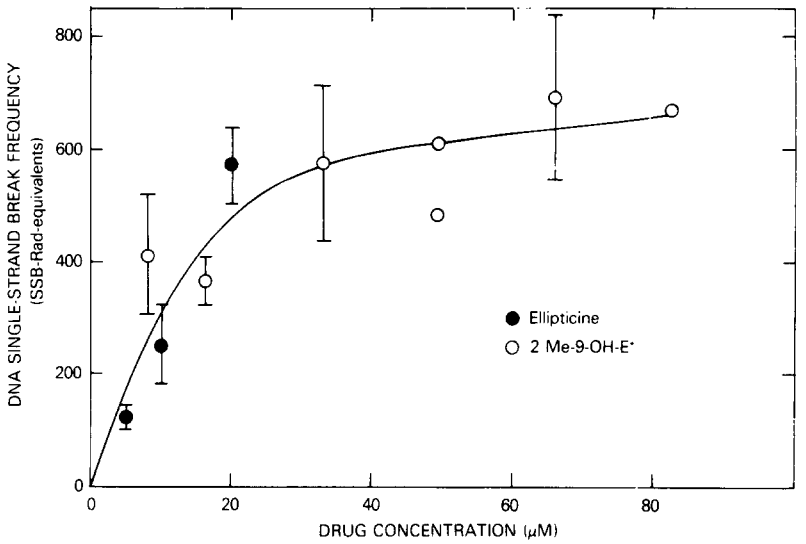

Fig. 3. DNA single-strand break frequency produced by various concentrations of ellipticine (●) or 2-Me-9-OH-E$^+$ (○). Cells were treated for 60 min at 37°. Single-strand break frequency was quantified by the alkaline elution technique using proteinase-K. Rad-equivalents indicate the dose of X-radiation that would give comparable elution in this assay. Pump speed for most experiments was 0.03 to 0.04 ml/min although a few experiments at 0.12 to 0.16 ml/min were included. Results in the two assays were consistent.

indicate two types of DNA breaks, one protein-associated and one not. All further studies with ellipticine were performed at doses producing solely protein-associated breaks ($\leq 20\ \mu M$).

Figure 5 shows that both compounds produced near equivalence of single-strand breaks and DNA–protein crosslinks. This equivalence has been demonstrated for several other intercalators [3, 4, 6, 7]. Table 1 lists the median, range, mean and standard deviation for the ratios of single-strand breaks to DNA–protein crosslinks. 2-Me-9-OH-E$^+$ gives a particularly good example of the equivalence for the two DNA effects.

DNA double-strand breakage can also be quantified using filter elution techniques [2, 6, 17, 18]. The results of pH 9.6 elution assays in drug-treated cells can be compared to results obtained from cells receiving various doses of X-radiation (in terms of

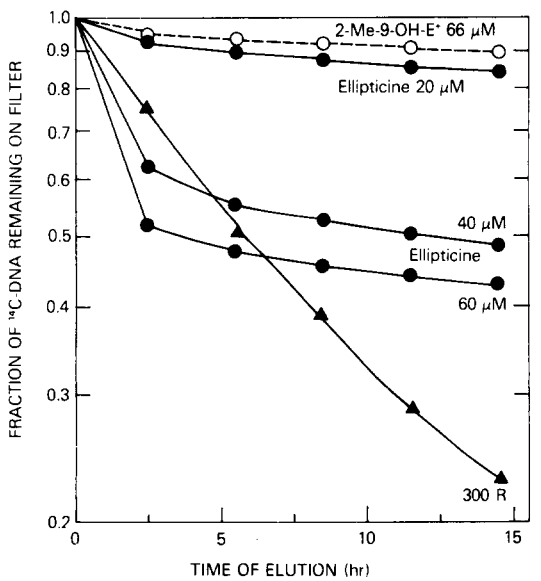

Fig. 4. Alkaline elution without the use of proteinase: 20, 40, or 60 μM ellipticine (●—●); 66 μM 2-Me-9-OH-E$^+$ (○—○); 300 rads X-ray (▲). L1210 cells were treated for 1 hr at 37° as in Figs. 2 and 3; pump speed, 0.03 to 0.04 ml/min.

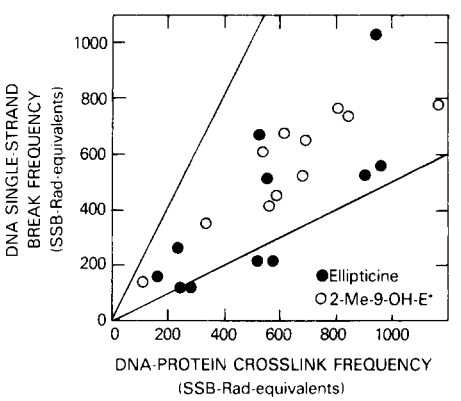

Fig. 5. Relationship between DNA single-strand break frequency and DNA–protein crosslink frequency in L1210 cells treated with ellipticine (●) or 2-Me-9-OH-E$^+$ (○). Single-strand breaks were quantified by the alkaline elution technique with proteinase (as in Fig. 3 legend). DNA–protein crosslinks were quantified in replicate aliquots using alkaline elution without proteinase (see Materials and Methods). Lines encompass area in which single-strand breaks and DNA–protein crosslinks approximate one another within a factor of 2.

Table 1. Relationship between DNA single-strand breaks (SSB) and DNA–protein crosslinks (DPC) or DNA double-strand breaks (DSB) produced by ellipticine and 2-Me-9-OH-E$^+$

	SSB/DPC*		[SSB]/[DSB]†	
	Median (range)‡	Mean ± S.D. (N)	Median (range)‡	Mean ± S.D. (N)
Ellipticine	0.58 (0.39–1.08)	0.73 ± 0.33 (11)	0.09 (0.04–0.14)	0.10 ± 0.05 (12)
2-Me-9-OH-E$^+$	0.94 (0.74–1.15)	0.95 ± 0.22 (11)	0.11 (0.08–0.14)	0.11 ± 0.3 (14)

* Ratio of SSB frequency: DPC frequency.

† [SSB] = rads of X-ray producing an equivalent DNA elution rate at pH 12.1. [DSB] = rads of X-ray producing an equivalent DNA elution rate at pH 9.6.

‡ Range encompassing 80% of values.

DSB-rad-equivalents) (see Materials and Methods and Ref. 6). These same drug-treated cells can also be used in the alkaline elution assay and single-strand breaks quantified and expressed in terms of the effect of X-rays (SSB-rad-equivalents). Thus, for any drug treatment a ratio of measured single-strand breaks to measured double-strand breaks can be obtained ([SSB]/[DSB]) and compared with the effects of X-radiation in the same assay. Literature values place the actual single- to double-strand break ratio for X-rays (k_{RS}/k_{RD}) between 10 and 40. We can compare this ratio (k_{RS}/k_{RD}) to the one measured for each intercalator ([SSB]/[DSB]) and through equation 4 obtain the actual single-strand break (excluding those arising from double-strand breaks) to double-strand break ratio (s/d) for the drugs. If only double-strand breaks were being produced by a drug, s would equal zero and measured [SSB]/[DSB] would be 0.05 to 0.2. This range is encompassed within the two lines in Fig. 6. Almost all the points fall within this range, and the median and mean values for this ratio with both drugs (Table 1) indicate the possibility that all of the ellipticine or 2-Me-9-OH-E$^+$ breaks are double-stranded. This was suggested previously for ellipticine by Ross and Bradley [2].

In Fig. 7, the rates of single-strand break production and disappearance are shown. Ellipticine produced breaks rapidly, and these breaks persisted following removal of cells from drug. By contrast, 2-Me-9-OH-E$^+$ produced breaks gradually with time and these breaks reversed following treatment with a half-time of disappearance approximating 30 min. As previously described for m-AMSA- and adriamycin-produced breaks [6], 2-Me-9-OH-E$^+$-induced breakage and reversal were temperature-dependent (Fig. 8). The gradual increase in break formation with time at 37° was not made more rapid by treating cells at 4° or 22° for 1 hr prior to shifting them to 37°. This indicates that the absence of break production or reversal at reduced temperature did not reflect cell death but, rather, the temporary inhibition of some cell process upon which DNA breakage depends. We have not, however, isolated the temperature-dependent step to that of actual

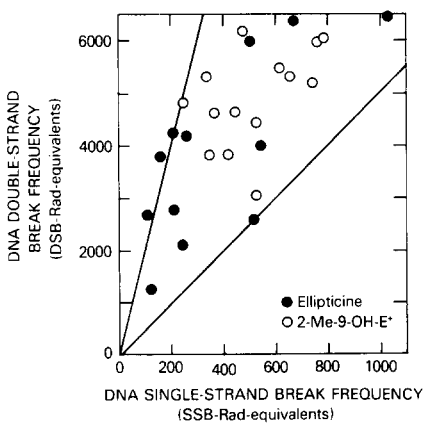

Fig. 6. Relationship between DNA double-strand breaks and DNA single-strand breaks in L1210 cells treated with ellipticine (●) or 2-Me-9-OH-E$^+$ (○). Double-strand breaks were quantified using the pH 9.6 filter elution method. Results are expressed in double-strand break (DSB) rad-equivalents which indicates the dose of X-radiation which would give comparable elution in this assay. Single-strand breaks in replicate aliquots were quantified and expressed in single-strand break (SSB) rad-equivalents as in the legend of Fig. 2 (see Materials and Methods). Lines encompass the area of uncertainty in which the ratio of measured double-strand breaks to single-strand breaks might fall if all of the breaks were double-stranded (see text for discussion).

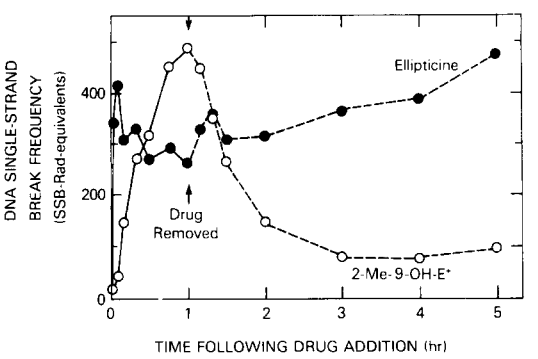

Fig. 7. Formation and disappearance of DNA single-strand breaks in L1210 cells treated with 10 μM ellipticine. (●) or 8.25 μM 2-Me-9-OH-E$^+$ (○). Cells were incubated with drug at 37° for various times up to 1 hr. Drug was removed from the medium by centrifugation and cells were resuspended in fresh medium (arrow). Single-strand breaks were quantified by alkaline elution at 0.03 to 0.04 ml/min using proteinase.

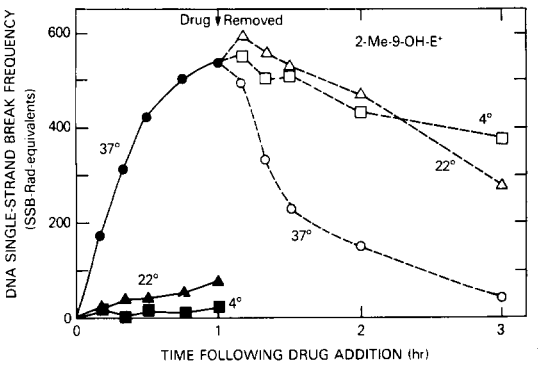

Fig. 8. Effect of reduced temperature on the formation and disappearance of DNA single-strand breaks in L1210 cells treated with 8.25 μM 2-Me-9-OH-E$^+$. Experiments were performed as described in the legend of Fig. 7 except that treatment was at 37° (●), 22° (▲) or 4° (■). Following a 1-hr treatment at 37°, cells were washed twice and incubated in fresh drug-free medium at 37° (--○--), 22° (--△--) or 4° (--□--).

break production rather than drug transport, metabolism or DNA intercalation. Finally, 2-Me-9-OH-E$^+$ DNA double-strand breakage mirrored single-strand breakage in the kinetics and temperature dependence of formation and disappearance (data not shown). This is as expected if all DNA breaks were double-stranded in nature (Fig. 6 and Table 1).

Representative intercalators from three chemical classes have now been found which produce reversible protein-associated DNA breaks. m-AMSA (acridines) [6], 5-iminodaunorubicin (anthracyclines) [7], and 2-Me-9-OH-E$^+$ (ellipticines) do, however, differ in the relative amount of double- to single-strand breakage produced. [DSB]/[SSB] ratios are, from highest to lowest, 2-Me-9-OH-E$^+$ > 5-iminodaunorubicin > m-AMSA. Figure 9 relates the magnitude of each type of DNA break for various drug doses to the associated cytotoxicity. The dependence of cytotoxicity on single- or double-strand break frequencies follows curves that are distinctly different for the three drugs. Further, the small difference in the half-time of break disappearance (m-AMSA, 5–10 min; 5-iminodaunorubicin, 20 min; 2-Me-9-OH-E$^+$, 30 min) cannot explain the variable associated cytotoxicity, since the relative degree of cytotoxicity does not mirror the relative degree of break persistence.

DISCUSSION

The mechanism by which intercalating agents kill cells is not known. The production by these agents of breaks in cellular DNA could potentially reflect a lethal lesion. A comparison between DNA effects and their associated cytotoxicity requires the treatment of cells for finite, measurable times, after which drug can be removed, DNA break production cease, and cell proliferation quantified. A comparison between m-AMSA, an acridine, and adriamycin, an anthracycline, could not be made because adriamycin persisted in cells and DNA break production continued following extracellular drug removal whereas m-AMSA exited from cells and the DNA breaks resealed [6]. The rapid reversal of the DNA effects of 5-iminodaunorubicin, an anthracycline, allowed us to compare an anthracycline and an acridine. No uniform relationship between DNA breakage and cytotoxicity was found in the comparison of 5-iminodaunorubicin and m-AMSA [7].

Ellipticines provide a third intercalator class for comparison. The parent compound, however, was similar to adriamycin in that it produced DNA breakage in cells following the removal of drug from the extracellular space (Fig. 7 and Ref. 8). A different ellipticine was needed which produced reversible

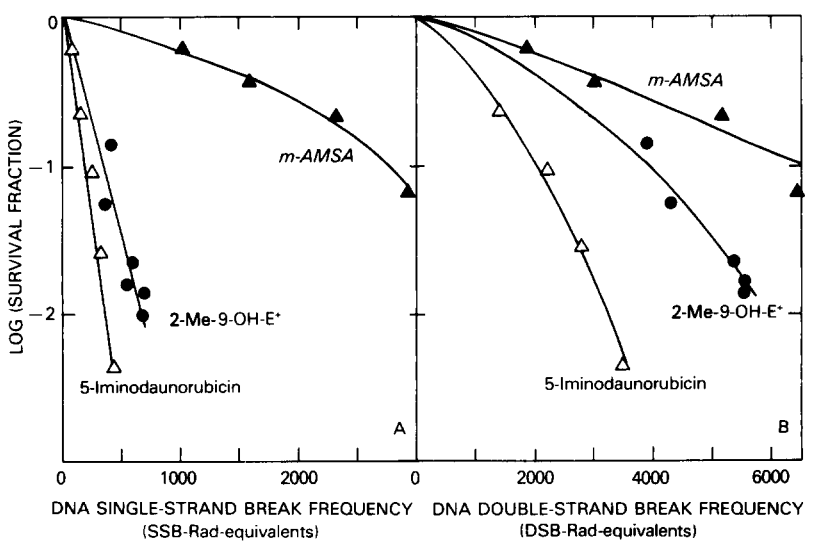

Fig. 9. Relationship between colony formation and DNA break frequency following 1-hr treatments with m-AMSA (▲), 5-iminodaunorubicin (△) or 2-Me-9-OH-E$^+$ (●). (A) Log survival fraction versus single-strand break frequency. (B) Log survival fraction versus double-strand break frequency.

DNA breakage if a comparison with the other intercalator classes was to be made.

2-Me-9-OH-E$^+$ provides an intercalator from the ellipticine class which produces reversible protein-associated DNA breaks that appear to be predominantly, if not entirely, double-stranded. The reversibility allows comparison between an ellipticine and intercalators of the acridine and anthracycline classes that also produce reversible, protein-associated breaks but differ in the ratio of single- to double-strand breaks produced. Comparisons of DNA effects and cytotoxicity produced by each type of drug revealed no uniform relationship (Fig. 9). Thus, representatives of three chemical classes of intercalators all produced apparently similar reversible, protein-associated DNA breaks which upon closer examination actually can be distinguished by their degrees of double-strandedness and their rates of reversal, but these differences are still inadequate to account for their different resultant cytotoxicities. It is possible that this DNA effect does not reflect a lethal event (i.e. DNA damage) but, rather, a cellular response to intercalation which involves an elicited binding of protein to DNA and a transient breaking of the DNA backbone.

Although ellipticine may produce the same effects as 2-Me-9-OH-E$^+$, its lack of reversibility impeded a comparison with other agents, as the actual duration of drug exposure could not be controlled. The persistence of DNA breaks following cell washing implied persistent intracellular drug. Ross and Smith [8] have recently demonstrated this persistence and have shown that it can be overcome by diluting cells in 100-fold excess volumes of medium. The difference between 2-Me-9-OH-E$^+$ and ellipticine probably stems from the greater lipophilicity of the latter, producing a depot of lipid- or membrane-associated drug [8–10].

The protein-associated DNA breakage produced by intercalating agents originally appeared to be a single effect. Differences among agents as to break formation and disappearance rates could be attributed to differences in drug uptake, metabolism or egress. The current results, as well as past results from this laboratory [6, 7] and from Ross and Bradley [2], indicate that a single effect cannot account for all intercalator-induced, protein-associated DNA breakage, since the relative amounts of single- and double-stranded breaks produced vary from drug to drug. Drugs from a single chemical class so far have produced similar [SSB]/[DSB] ratios (e.g. adriamycin and 5-iminodaunorubicin, ellipticine and 2-Me-9-OH-E$^+$) (Ref. 7 and Fig. 6). Ellipticines, and 2-Me-9-OH-E$^+$ in particular, will be important compounds in future work as they may produce predominantly double-strand breaks. If, as we have previously hypothesized, intercalator-induced breaks arise from an enzymatic, cellular response to DNA intercalation and at least two responses are possible, one resulting in single-strand scissions and one in double-strand scissions, then ellipticines may elicit predominantly the double-strand break response, thus potentially allowing the isolation of the causative protein(s). 2-Me-9-OH-E$^+$ will be particularly useful as it produced solely protein-concealed breaks even at doses producing large magnitudes of cell killing. The reversibility and temperature sensitivity of the 2-Me-9-OH-E$^+$ break effect also will be useful in future studies of the origin of intercalator-induced DNA scission.

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