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DNA Minor Groove Binding Agents Interfere with Topoisomerase II Mediated Lesions Induced by Epipodophyllotoxin Derivative VM-26 and Acridine Derivative *m*-AMSA in Nuclei from L1210 Cells†

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ABSTRACT: This study demonstrated that agents capable of interacting with the minor groove in nuclear DNA interfere with topoisomerase II mediated effects of antitumor drugs such as VM-26 and *m*-AMSA. Distamycin, Hoechst 33258, and DAPI were used as agents capable of AT-specific binding in the minor groove of DNA while producing no profound long-range distortion of DNA structure. In intact nuclei from L1210 cells, these minor groove binders inhibited the induction of topoisomerase II mediated DNA damage (DNA–protein cross-links and DNA double-strand breaks) by VM-26 and *m*-AMSA. The inhibitory effects of distamycin reflected prevention of formation of new lesions but not reversal of preexisting damage. The minor groove binders did not differentiate between lesions induced by an intercalator, *m*-AMSA, or by a DNA-nonbinding drug, VM-26. All three groove binders inhibited DNA breaks more strongly than DNA–protein cross-links. The inhibitory potency correlated with the size of minor groove binders and the size of their DNA-binding sites: distamycin (5 bp) > Hoechst 33258 (4 bp) > DAPI (3 bp). The results showed that DNA minor groove binders are a new type of modulators of the action of topoisomerase II targeted drugs.

DNA topoisomerase II has attracted considerable attention recently as a new target for antitumor drugs [for review see Ross (1985) and Wang (1987)]. This important enzyme controls topology of cellular DNA and plays a role in vital cellular processes such as replication, transcription, and mitosis (Brill et al., 1987; Liu & Wang, 1987; Snapka et al., 1988; Uemura et al., 1987). In the course of its reaction, topoisomerase II introduces a transient double-strand break in the DNA and binds covalently to 5′ ends of the broken strands (Ross, 1985; Wang, 1987; Tewey et al., 1984; Chen et al., 1984). This reaction intermediate is referred to as the "cleavable complex". A number of antitumor drugs trap the enzyme at this stage, preventing the restoration of intact DNA structure (Ross, 1985; Wang, 1987). Drug-induced stabilization of the cleavable complexes can be monitored in whole cells or nuclei as induction of DNA–protein cross-links accompanied by DNA double-strand breaks. The formation of these lesions correlates with antiproliferative effects of topoisomerase II targeted antitumor drugs (Ross, 1985; Wang, 1987).

Drugs stabilizing cleavable complexes of topoisomerase II belong essentially to two groups. The first group consist of drugs capable of binding to DNA by intercalation. A representative agent of this group is the acridine derivative *m*-AMSA (Ross, 1985; Tewey et al., 1984). However, interca-

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lation is not sufficient for this effect. For example, *o*-AMSA, which is a better intercalator than *m*-AMSA, is unable to trap the cleavable complex (Tewey et al., 1984). Another class of drugs comprises agents that stabilize cleavable complexes but do not bind to DNA. This group includes the epipodophyllotoxin congeners VM-26 and VP-16 (Ross, 1985; Chen et al., 1984). It is not known whether various types of drugs stabilize cleavable complexes by common or distinct pathways. Several other aspects of their action also remain obscure. For example, protein-associated breaks (which reflect stabilization of cleavable complexes) are readily reversible while drug cytotoxicity is not (Tewey et al., 1984; Chen et al., 1984; Pommier et al., 1985a,b). Such gaps in the knowledge of drug action hamper mechanism-based development of antitopoisomerase agents as clinical drugs.

The approach we have chosen to learn more about drug interference with topoisomerase II is to probe the drug action by using other agents that bind to the DNA in a defined manner. Compounds useful as probes should (i) lack the ability to stabilize cleavable complexes themselves and (ii) induce only local alterations to the DNA. Preference for specific DNA sequences as well as demonstrated potential to interfere with topoisomerase II or other DNA-processing enzymes would also be desirable.

Thus far, only agents intercalating between DNA bases, such as ethidium bromide or *o*-AMSA, have been shown to inhibit DNA lesions induced by topoisomerase II targeted drugs (Rowe et al., 1987). These inhibitory effects are related to the ability of intercalators to impede the catalytic activity of topoisomerase II (Tewey et al., 1984; Pommier et al., 1987). However, intercalators unwind DNA, which leads to long-range changes in the DNA structure (Neidle, 1987). Therefore, the interpretation of the results for this class of DNA binders is complex and limited (Pommier et al., 1987).

In contrast to the intercalators, compounds binding on the outside of the DNA in its minor groove generally produce very little or no long-range distortion of the helix (Neidle, 1987). Moreover, no agent of this class has been found to stabilize the cleavable complexes. Minor groove binders (e.g., distamycin and netropsin) can affect the actions of other DNA-processing enzymes, for example, RNA polymerases (Bruzik et al., 1987). Recently, we have shown that the AT-specific minor groove binders, the antibiotic distamycin (Fish et al., 1988; Coll et al., 1987) and the fluorescent stains Hoechst 33258 (Teng et al., 1988) and DAPI (Manzini et al., 1986) (Figure 1), interfere with the catalytic activity of isolated topoisomerase II (Woynarowski et al., 1989) and topoisomerase I (McHugh et al., 1989).

In this study, we have investigated the potential of these compounds to modulate DNA lesions induced in nuclei by the topoisomerase II targeted drugs VM-26 and *m*-AMSA. All three groove binders, distamycin, Hoechst 33258, and DAPI, were found to strongly inhibit DNA–protein cross-links and DNA double-strand breaks induced by both classes of antitumor drugs. These results show a new approach to the modulation of topoisomerase II targeted drugs.

Materials and Methods

Drugs and Biochemicals. Distamycin and 4′,6-diamidino-2-phenylindole (DAPI) were obtained from Sigma, St. Louis, MO. 2-(4-Hydroxyphenyl)-5-[5-(4-methylpiperazin-1-yl)-benzimidazol-2-yl]benzimidazole (Hoechst 33258) was from Aldrich. The stock solutions of the minor groove binders were made in water and stored at −20 °C. VM-26 was a generous gift from Dr. W. Bradner at Bristol-Myers, Wallingford, CN, while 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) was provided by Warner Lambert Pharmaceutical Co., Ann Arbor, MI. The stock solutions of VM-26 and *m*-AMSA were made in DMSO and stored at −20 °C.

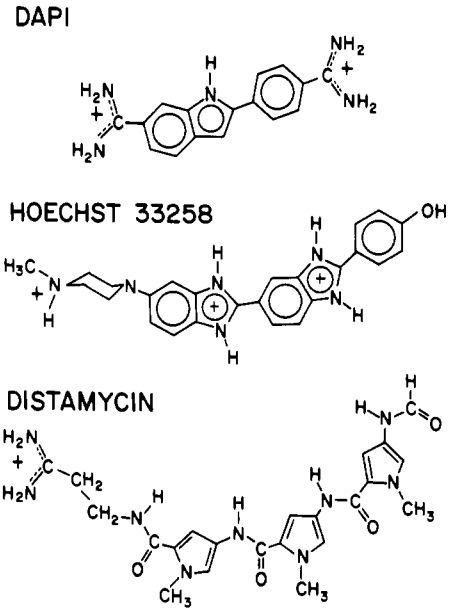

FIGURE 1: Structures of minor groove binders.

Cell Labeling and Nuclei Isolation. Leukemia L1210 cells were grown as described (Woynarowski et al., 1988) and labeled with 0.06–0.1 μCi/mL [2-^{14}C]thymidine (56 Ci/mmol, Moravek Biochemicals, Brea, CA) for 24–36 h. Before nuclei isolation or drug treatment, the cells were incubated for 30 min in fresh medium without label. The nuclei were isolated according to Glisson et al. (1984). Isotonic conditions were employed in this procedure to prevent the disruption of higher order chromatin structures (Walker et al., 1986). Isolated nuclei were resuspended in 2 mM KH_2PO_4, 5 mM $MgCl_2$, 150 mM NaCl, and 1 mM EGTA (pH 6.9) at 0.5×10^6 nuclei/mL.

Drug Treatment of Nuclei. Aliquots of nuclei suspension were supplemented with 0.4 mM ATP and treated with the drugs. Unless indicated otherwise, antitopoisomerase II drugs were routinely added to nuclei immediately before the addition of minor groove binding agents followed by incubation at 37 °C for 30 min. However, similar results were obtained when a minor groove binder was added first followed by addition of VM-26 or *m*-AMSA after 1-min incubation at 37 °C. After treatment, 0.5×10^6 and 0.3×10^6 nuclei were analyzed for DNA–protein cross-links and DNA double-strand breaks, respectively. Parallel samples were used for the determination of total radioactivity as described (Woynarowski et al., 1988). Typically, total radioactivity amounted to about 10^5 cpm/10^6 nuclei.

DNA–Protein Cross-Links. DNA linked covalently to protein was assayed by coprecipitation with potassium dodecyl sulfate as described previously (Woynarowski et al., 1988) except that DNA was sheared only by vortexing at full speed for 15 s instead of sonication. The results are expressed as fraction of total DNA coprecipitating with protein.

DNA Double-Strand Breaks. DNA breakage was monitored by filter elution under nondenaturing conditions (Bradley & Kohn, 1979) using a low-sensitivity procedure as described previously (Beckman et al., 1987). The samples of nuclei after drug treatment were lysed on polycarbonate filters by incubation with sodium dodecyl sulfate (2%) and proteinase K (0.25 mg/mL) in 25 mM EDTA for 1 h at 37 °C followed by 30 min at 20 °C. DNA was eluted from the filter with

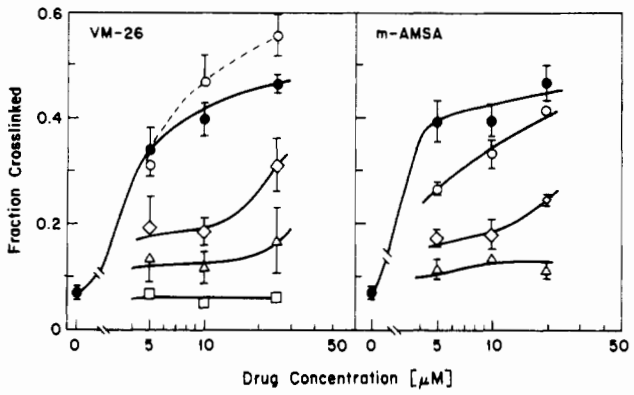

FIGURE 2: DNA–protein cross-links induced in L1210 nuclei by varying concentrations (indicated on the *x* axis) of VM-26 (left panel) or *m*-AMSA (right panel) in the absence (●) and the presence of distamycin at 5 μM (○), 10 μM (◇), 25 μM (△), and 50 μM (□). Nuclei were incubated with the drugs for 30 min at 37 °C followed by determination of DNA–protein cross-links by potassium dodecyl sulfate precipitation as described under Materials and Methods. Error bars correspond to the SEM from two to three experiments carried out in duplicate.

2% tetrapropylammonium hydroxide, 0.1% sodium dodecyl sulfate, and 20 mM EDTA, pH 9.7.

RESULTS

On the basis of our previous finding that minor groove binders are able to alter the catalytic activity of isolated topoisomerase II (Woynarowski et al., 1989), we questioned whether these agents could interfere with topoisomerase II mediated effects of antitumor drugs. This study determined the effects of minor groove binders on DNA lesions induced in nuclei by either of two topoisomerase-targeted drugs, VM-26 or *m*-AMSA. These drugs represent two basic types of agents capable of stabilizing cleavable complexes: DNA-nonbinding drugs and DNA intercalators. The same groove binders, distamycin, Hoechst 33258, and DAPI (Figure 1), that interfered with the isolated enzyme (Woynarowski et al., 1989) were studied as potential modulators.

DNA–Protein Cross-Links. The initial experiments assessed the effects of distamycin on DNA–protein cross-links induced in L1210 nuclei treated with VM-26 and *m*-AMSA using the technique of potassium dodecyl sulfate coprecipitation of DNA covalently bound to protein. Distamycin, as well as DAPI or Hoechst 33258, alone were unable to induce any DNA–protein cross-links in nuclei (data not shown). Thus these agents were unable to stabilize the cleavable complexes.

In the absence of distamycin, both topoisomerase II targeted drugs produced a substantial fraction of cross-linked DNA (Figure 2). Addition of distamycin at 10–50 μM resulted in a potent, dose-dependent inhibition of VM-26-induced cross-links. At a higher concentration of VM-26 (resulting in greater cross-linking in the absence of distamycin), the extent of inhibition was somewhat decreased except for 50 μM distamycin, which completely abolished the formation of DNA–protein cross-links (Figure 2). Similar inhibition by distamycin was noted with DNA–protein cross-links induced by *m*-AMSA (Figure 2).

The lowest distamycin concentration (5 μM) produced a small enhancement of VM-26-induced cross-links (Figure 2). This effect is consistent with previously observed stimulation of topoisomerase II catalytic activity by low concentrations (1–5 μM) of distamycin (Woynarowski et al., 1989).

The time dependence of distamycin's inhibitory effect was examined to see whether it reflected the prevention of DNA lesion formation. In Figure 3A, the effect of VM-26 alone

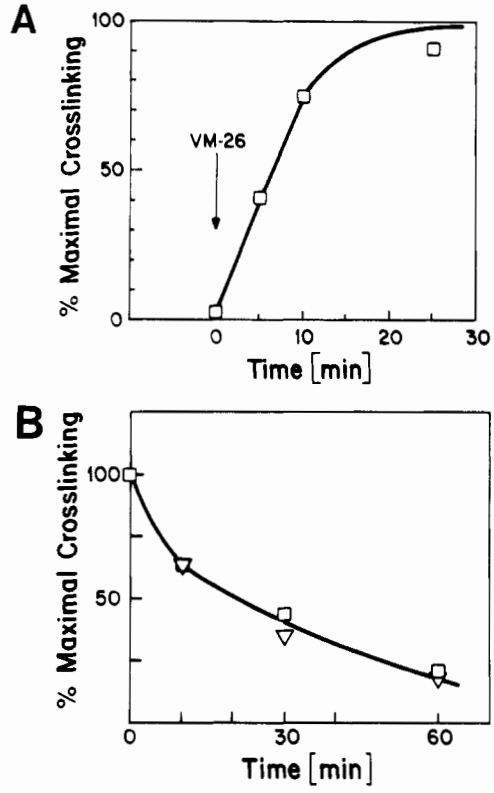

FIGURE 3: (A) Inhibition of VM-26-induced DNA–protein cross-links as a function of time of addition of distamycin. L1210 nuclei were incubated with VM-26 (10 μM), and distamycin (25 μM) was added at the times indicated. Incubation with VM-26 was for 30 min at 37 °C. The levels of DNA–protein cross-links in these samples are expressed as percent of cross-links detected in the absence of distamycin. (B) Lack of distamycin effect on the reversal of DNA–protein cross-links induced by VM-26. L1210 nuclei were incubated with VM-26 (10 μM) for 30 min at 37 °C. Next, the nuclei were centrifuged (1200 rpm, 7 min), resuspended in fresh buffer without any drug (□) or with 25 μM distamycin (▽), and further incubated at 37 °C as indicated.

(100%) is compared to DNA–protein cross-links remaining when distamycin was added at various times after addition of VM-26. There was a complete inhibition of cross-link formation when distamycin (25 μM) was added together with VM-26. However, when the minor groove binder was added after VM-26 addition, the inhibition was gradually reduced. Most of cross-links induced by VM-26 in the absence of distamycin are formed during the initial 15 min (data not shown). These data indicated that the lesions formed before the addition of distamycin were not affected by the groove binder although the formation of new lesions was aborted.

Topoisomerase II mediated DNA lesions are rapidly reversible following removal of VM-26 from the media (Pommier et al., 1985a,b). Another experiment assessed distamycin's ability to affect this spontaneous reversal (Figure 3B). The addition of distamycin (at 25 μM) to nuclei resuspended in fresh buffer after the incubation with VM-26 had no effect on the rate of disappearance of VM-26-induced DNA–protein cross-links (Figure 3B).

Hoechst 33258 and DAPI were tested to see if the ability to inhibit stabilization of the cleavable complex is a common feature for groove binders which inhibit catalytic activity of topoisomerase II. A composite graph comparing all three minor groove binders is shown in Figure 4. Both, Hoechst 33258 and DAPI are also able to inhibit DNA–protein cross-links induced by both VM-26 or *m*-AMSA in a concentration-dependent manner. Hoechst 33258, DAPI, and distamycin, however, differed in their ability to inhibit

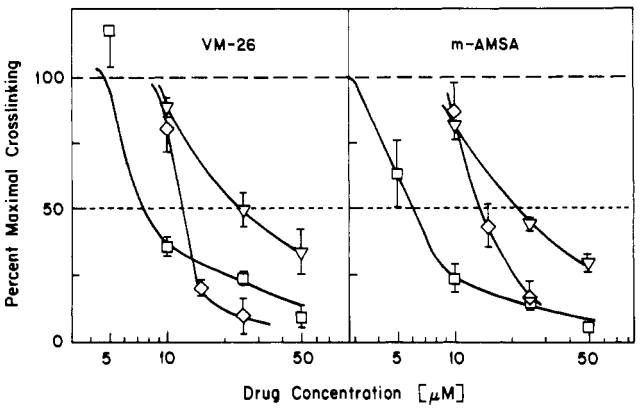

FIGURE 4: Inhibition of DNA–protein cross-links induced in L1210 nuclei by VM-26 (10 μM) or *m*-AMSA (20 μM) by varying concentrations of distamycin (□), Hoechst 33258 (◇), or DAPI (▽). Maximal cross-linking (100%) corresponds to the effect of VM-26 or *m*-AMSA alone. For other details see Figure 1 and Materials and Methods. Error bars correspond to the SEM from two to three experiments carried out in duplicate.

cross-link formation. In combination with VM-26, distamycin showed inhibitory properties at slightly lower concentrations than Hoechst 33258 while DAPI was considerably less active (Figure 4). The same pattern was noted with *m*-AMSA.

DNA Double-Strand Breaks. The effects of the minor groove binders on DNA–protein cross-links were presumed to be a result of the interference with the stabilization of the cleavable complexes. In subsequent experiments, we determined whether DNA double-strand breaks, the second type of DNA lesion that is mediated by topoisomerase II, were similarly affected. Double-strand breaks were assayed by the technique of neutral elution.

The results of these experiments are shown in Figure 5. In the absence of distamycin, VM-26 at 10 μM induced a substantial amount of damage. Approximately 65% of DNA eluted from the filters after a short elution time (55 min), whereas only a very small fraction was eluted from control samples. Addition of 10 μM distamycin to nuclei profoundly decreased the DNA breakage (Figure 5A). At the highest concentration of this agent (25 μM), almost complete inhibition was observed.

Table I: Comparison of the Ability of Distamycin, Hoechst 33258, and DAPI To Inhibit DNA Lesions Induced in L1210 Cell Nuclei by VM-26 (at 10 μM) or *m*-AMSA (at 20 μM)

	C_{50} (μM)[a]	
	VM-26	*m*-AMSA
DNA–Protein Cross-Links		
distamycin	8	6
Hoechst 33258	12	15
DAPI	25	22
DNA Double-Strand Breaks		
distamycin	6	3
Hoechst 33258	9	ND[b]
DAPI	13	≪25[c]

[a] C_{50} is the concentrations of minor groove binder inhibiting formation of DNA–protein cross-links or DNA double-strand breaks by 50%. [b] Not determined. [c] 100% inhibition at 25 μM DAPI.

The other minor groove binders, Hoechst 33258 and DAPI, also inhibited the induction of DNA double-strand breaks by VM-26. The inhibitory effects of Hoechst 33258 and DAPI are compared to the effect of distamycin (Figure 5B). These results are very similar to the data on inhibition of DNA–protein cross-links (cf. Figure 2B). Again, distamycin appeared to be the most potent inhibitor, followed by Hoechst 33258 and then DAPI. Neither distamycin, Hoechst 33258, nor DAPI alone influenced the elution of nuclear DNA (data not shown). This observation confirms that these groove binders cannot themselves stabilize the cleavable complexes.

The effect of distamycin on DNA breaks induced by *m*-AMSA was also investigated. The latter drug is a much weaker inducer of DNA double-strand breaks than VM-26 (Figures 6A and 5A, respectively) when compared at concentrations producing an approximately identical amount of DNA–protein cross-links (cf. Figure 2). Nonetheless, distamycin was able to inhibit *m*-AMSA-induced breaks in a concentration-dependent manner (Figure 6). Thus, this effect resembled the inhibition of VM-26-induced lesions.

Comparison of the Effects of Distamycin, Hoechst 33258, and DAPI on DNA Lesions Induced by VM-26 and m-AMSA. The effects of all three minor groove binders on both types of DNA lesions are summarized in Table I. To compare these effects, the results are expressed as the concentrations of minor

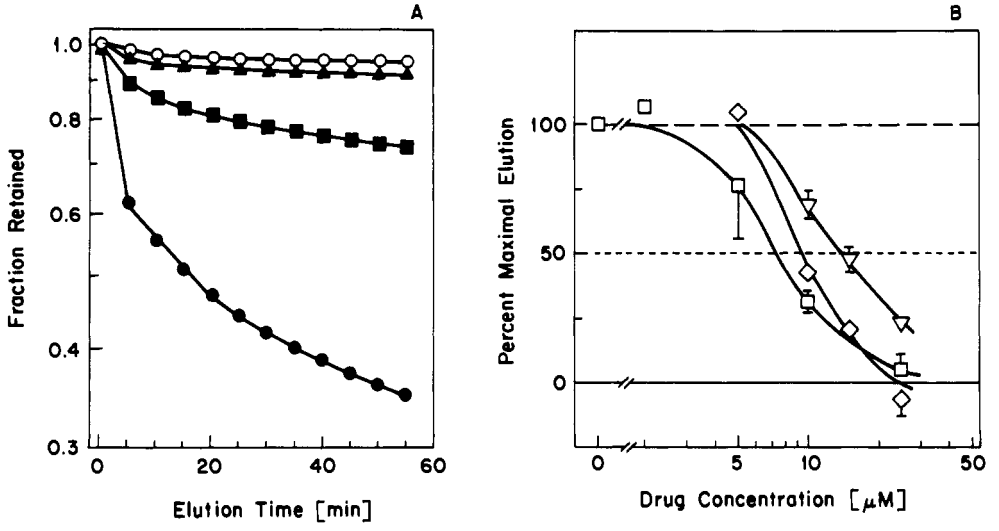

FIGURE 5: Effects of minor groove binders on DNA double-strand breaks induced in L1210 nuclei by VM-26 (10 μM) as determined by the filter elution technique. (A) Elution profiles of DNA from control nuclei incubated in the absence of drugs (○), and from nuclei incubated with 10 μM VM-26 alone (●) or 10 μM VM-26 and 10 μM (■) or 25 μM (▲) distamycin. (B) Inhibition of DNA double-strand breaks induced in L1210 nuclei by VM-26 (10 μM) by varying concentrations of distamycin (□), Hoechst 33258 (◇), or DAPI (▽). Maximal elution (100%) corresponds to DNA cleavage by VM-26 alone. Error bars correspond to the SEM from two to three experiments. For other details see Materials and Methods.

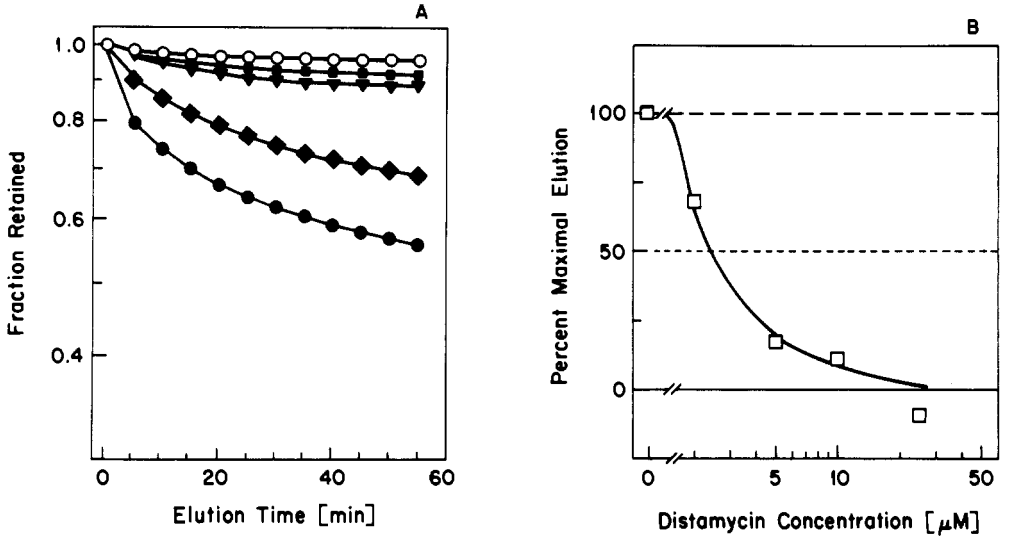

FIGURE 6: Effects of distamycin on DNA double-strand breaks induced in L1210 nuclei by *m*-AMSA (20 μM) as determined by the filter elution technique. (A) Elution profiles of DNA from control nuclei incubated in absence of drugs (○), and from nuclei incubated with 20 μM *m*-AMSA alone (●) or 20 μM *m*-AMSA with distamycin at 2 μM (♦), 5 μM (▼), and 10 μM (■). (B) Inhibition of DNA double-strand breaks induced in L1210 nuclei by *m*-AMSA (20 μM) by varying concentrations of distamycin. Maximal elution (100%) corresponds to DNA cleavage by *m*-AMSA alone. For other details see Materials and Methods.

groove binders inhibiting by 50% the formation of DNA lesions (C_{50}). The minor groove binders did not differentiate in their inhibitory action between DNA–protein cross-links induced by VM-26 and *m*-AMSA since the C_{50} values were very close for both antitopoisomerase II drugs. The C_{50} values for DNA–protein cross-links also indicated that distamycin was more potent than Hoechst 33258, while DAPI was clearly the least active. The same order of activity was seen for DNA breaks. The latter lesion, however, seemed to be more sensitive to inhibition than DNA–protein cross-links. The fact that such a close relation was seen for both types of DNA lesions and both classes of antitopoisomerase II drugs strengthens the interpretation that the inhibitory effects of the minor groove binders are, indeed, indicative of disturbed action of topoisomerase II.

The lesions induced in nuclei by VM-26 and *m*-AMSA occur preferentially in nascent DNA, and such a specific damage may be crucial for drug cytotoxicity (Woynarowski et al., 1988). These lesions can also be modulated by minor groove binders as we found that distamycin inhibited DNA–protein cross-links induced in nascent DNA by VM-26 (data not shown).

Effects of Hoechst 33258 in Intact L1210 Cells. An important question was whether minor groove binders interfere with the induction of cleavable complexes in whole cells. Using Hoechst 33258 as an agent that is reasonably well taken up by cells, we showed that the effects of a groove binder in intact cells resemble those in nuclei. Hoechst 33258 at 50 μM produced a significant reduction in the level of VM-26-induced DNA–protein cross-links (Figure 7A). As in nuclei, an even greater inhibitory effect of the groove binder was seen for DNA double-strand breaks induced by VM-26. Approximately 60% inhibition of DNA cleavage was observed for 50 μM Hoechst 33258 (Figure 7B).

Discussion

This study demonstrates that DNA lesions induced by VM-26 and *m*-AMSA can be inhibited by the DNA minor groove binders distamycin, Hoechst 33258, and DAPI. These effects were primarily characterized in intact nuclei in which drug-induced DNA lesions were mediated by endogenous topoisomerase II. By use of Hoechst 33258, similar effects were also shown in whole cells. The effects observed in nuclei and cells are consistent with the previously described modulation by minor groove binders of the catalytic activity of isolated topoisomerase II (Woynarowski et al., 1989). The inhibitory effects of distamycin result from the prevention of formation of new lesions, not reversal of preexisting lesions (i.e., those generated before the addition of a groove binder). These findings represent the first example of modulation of topoisomerase II targeted drugs by agents binding to the minor groove in DNA.

The minor groove binders inhibited two types of DNA lesions, DNA–protein cross-links and DNA double-strand breaks. While both types of lesions reflect presumably the same effect, stabilization of cleavable complexes of topoisomerase II by VM-26 and *m*-AMSA, they are not equally affected. For all three minor groove binders, DNA breakage was inhibited more strongly than DNA–protein cross-links. At present, we cannot exclude a greater sensitivity of the elution assay for DNA breaks as compared to DNA–protein cross-links determination by potassium dodecyl sulfate precipitation. However, it is also possible that some lesions do not reflect cleavable complexes but rather other mechanisms refractory to the inhibition by minor groove binders. For example, epipodophyllotoxins are able to bind covalently to DNA and protein after metabolic activiation (Haim et al., 1987). Likewise, *m*-AMSA can directly damage DNA without mediation of topoisomerase II (Wong et al., 1986).

The mechanisms by which a DNA-nonbinding agent (VM-26) and an intercalator (*m*-AMSA) trap cleavable complexes of topoisomerase II might be different. Therefore, the response of both drugs to the modulation might also differ. However, distamycin, Hoechst 33258, and DAPI had similar effects on DNA lesions induced by *m*-AMSA and VM-26. This suggests that the groove binders affect a stage in the formation of DNA lesions that is common for both types of topoisomerase-targeted drugs. As previously mentioned, distamycin, Hoechst 33258, and DAPI are each able to impede the catalytic activity of isolated topoisomerase II (Woynarowski et al., 1989). Both inhibition of enzyme activity and interference with drug-induced lesions in nuclei occur at similar drug concentrations. Both effects are likely to originate from specific local alterations in the minor groove which may result in a diminished

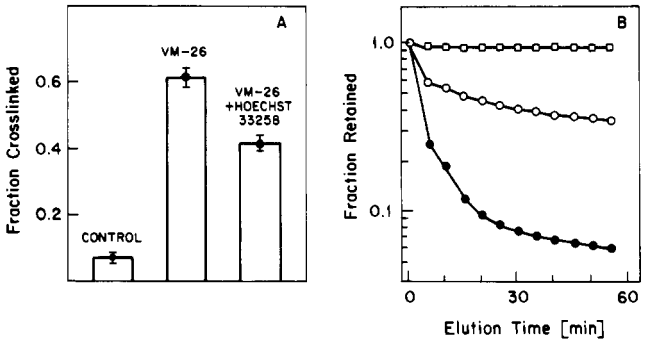

FIGURE 7: Effects of Hoechst 33258 on DNA–protein cross-links (panel A) and DNA double-strand breaks (panel B) induced in intact L1210 cells by VM-26. Cells were incubated with Hoechst 33258 (50 μM) for 60 min at 37 °C followed by addition of VM-26 (10 μM) and further incubation for 30 min. The cells were washed with PBS and analyzed for DNA–protein cross-links and DNA double-strand breaks as described under Materials and Methods for nuclei. Hoechst 33258 alone did not induce any DNA–protein cross-links or DNA breaks (data not shown). (A) DNA–protein cross-links: the results represent average values (±SEM) from five experiments carried out in duplicate. The reduction of VM-26-induced cross-links by Hoechst 33258 was highly significant ($p < 0.01$) as found by paired t test. Fraction cross-linked in the presence of Hoechst 33258 alone did not differ from that for control. (B) Elution profiles of DNA from control cells incubated in absence of drugs (□), and from cells incubated with 10 μM VM-26 alone (●) or 10 μM VM-26 with Hoechst 33258 at 50 μM (○).

accessibility of topoisomerase II reaction sites to the enzyme (Woynarowski et al., 1989). Such an interpretation is further corroborated by parallel studies in which we demonstrated that distamycin, Hoechst 33258, and DAPI can inhibit isolated topoisomerase I (McHugh et al., 1989) and DNA lesions that are induced in nuclei by camptothecin, a topoisomerase I targeted drug (McHugh et al., unpublished data).

The ability of the minor groove binders to inhibit both DNA–protein cross-links and DNA breaks correlates with their length (cf. Figure 1) and size of DNA-binding sites: distamycin [5 base pairs (Coll et al., 1987)] > Hoechst 33258 [4 base pairs (Teng et al., 1988)] > DAPI [3 base pairs (Manzini et al., 1983)]. The same order of activity was found in the case of topoisomerase I mediated lesions induced by camptothecin (McHugh et al., unpublished data). These correlations lend further support to the interpretation that the observed effects are due to specific, local interaction of a minor groove binder with the DNA. Overall, the results suggest that the minor groove may be involved in the action of topoisomerase II and drugs that stabilize the cleavable complexes. Interestingly, a recent study of *m*-AMSA–DNA complexes proposed that the drug's aniline side chain is located in the minor groove of AT sequences while the chromophore intercalates into DNA (Chen et al., 1988). It has been suggested that this side chain is responsible for the interaction with topoisomerase II which leads to the stabilization of the cleavable complexes (Chen et al., 1988). Thus, binding of distamycin, Hoechst 33258, or DAPI to AT sequences in the minor groove would disturb the interaction between *m*-AMSA and the enzyme.

To summarize, this investigation demonstrated the potential usefulness of DNA minor groove binders as probes and modulators of the action of topoisomerase II targeted drugs. Additional studies are needed to determine the effects of other groove binders and the relevance of the interference with drug-induced lesions to cytotoxicity of topoisomerase-targeted drugs. In the future, such studies might be helpful in providing leads for the mechanism-based development of promising new drug combinations.

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