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EFFECTS OF MINOR GROOVE BINDING DRUGS ON CAMPTOTHECIN-INDUCED DNA LESIONS IN L1210 NUCLEI

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Abstract—Topoisomerase I inhibition detected in mammalian cells can be correlated with reduced tumor growth. Camptothecin specifically inhibits topoisomerase I by stabilization of a covalently linked DNA–enzyme complex and associated DNA single-strand breaks. Whether perturbations in nuclear DNA structure can alter camptothecin-induced DNA damage was examined using the non-intercalative DNA minor groove binders distamycin, Hoechst 33258 and DAPI (4′,6-diamidino-2-phenylindole). L1210 nuclei were treated with camptothecin alone or in the presence of single minor groove binders. DNA–protein crosslinks and single-strand breaks were determined using potassium–sodium dodecyl sulfate precipitation and alkaline elution respectively. Distamycin produced a dose-dependent decrease in DNA–protein crosslinks and strand breaks. This effect was reduced if nuclei were treated with camptothecin prior to distamycin addition. Distamycin was unable to reverse lesions once induced or to prevent repair of damage upon camptothecin removal. Hoechst 33258 and DAPI also decreased camptothecin-induced DNA damage. The order of inhibitory potency was: distamycin > Hoechst > DAPI. This order corresponded to the molecular weights as well as to the size of the nucleotide binding sites of the drugs. Identifying agents which alter such DNA lesions should provide better understanding of the chemotherapeutic activity of camptothecin as well as help elucidate new leads for drug combinations of improved therapeutic benefit.

The alkaloid camptothecin (Fig. 1), originally purified from *Camptotheca acuminata* [1], shows antitumor activity in experimental tumor systems as well as in humans [2–4]. The cytotoxicity of the drug has been correlated with its ability to inhibit DNA and RNA synthesis [4] as well as to induce DNA fragmentation [5, 6]. Recently, the cellular target of camptothecin was defined as topoisomerase I [7]. Camptothecin inhibits topoisomerase I via formation of a camptothecin–topoisomerase I–DNA "cleavable complex" resulting in DNA single-strand breaks and DNA–protein crosslinks [8]. The presence of this complex has been correlated with camptothecin cytotoxicity [9]. While the precise nature of the complex remains unclear, treatment with protein denaturants indicates formation of a covalent bond between the topoisomerase I protein and DNA [8].

Despite current interest in topoisomerase I as a chemotherapeutic target [10], surprisingly few studies have been directed at defining factors which modulate formation of the cleavable complex. Such factors should include direct interference with the camptothecin–topoisomerase I–DNA complex, changes in enzyme specificity or activity, or alterations in DNA structure. Limited evidence for each type of modulation is available. Disruption of cleavable complex itself can be potentiated with high salt or increased temperature [7, 8]. Such treatments promote reversal of camptothecin strand breaks both in intact cells and in isolated enzyme preparations. At the enzyme level, changes in topoisomerase I in camptothecin-resistant mammalian cells mutants have also been described [11]. Topoisomerase I isolated from certain of these mutants is 10-fold less sensitive to camptothecin than enzyme from the parent strain. A few reports suggesting modulation of cleavable complex by means of DNA structural alteration are also available. For example, specific nucleotide sequences are required for topoisomerase I cleavage activity both in the absence and in the presence of camptothecin [12]. Additional effects of DNA modifications on camptothecin reactivity can be inferred from studies of topoisomerase I alone. DNA intercalating agents have been shown to inhibit topoisomerase I mediated DNA relaxation [13]. This inhibition correlated directly with the ability of the intercalators to unwind DNA. A recent report from this laboratory showed that the non-intercalative DNA minor groove binding drugs distamycin, DAPI and Hoechst 33258 (Fig. 2) have similar effects on topoisomerase I activity [14]. However, whether these or other DNA reactive agents also modulate the camptothecin–topoisomerase I interaction has not been established.

Camptothecin

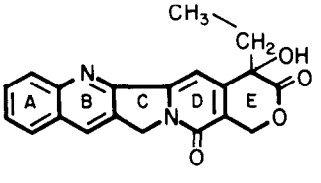

Fig. 1. Chemical structure of camptothecin.

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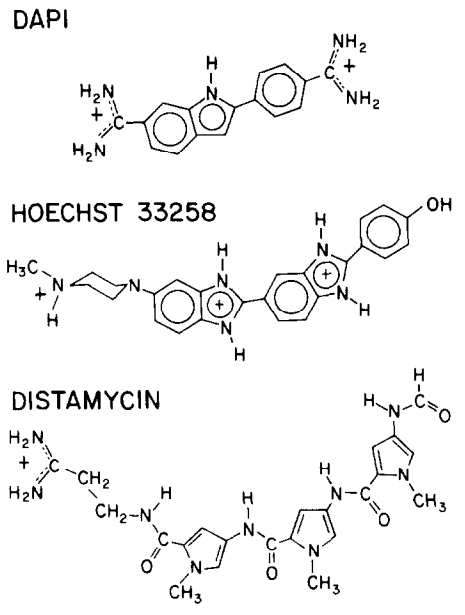

Fig. 2. Structures of three minor groove binding agents: DAPI, Hoechst 33258, and distamycin.

Minor groove binding drugs do not intercalate, nor do they distort the helix [15]. Rather, they bind to the outside of the minor groove by hydrogen bonds and hydrophobic interactions. Their primary effect is a widening of the minor groove at the drug binding site (generally, runs of A-T base pairs [15]). Besides the aforementioned alteration in topoisomerase I activity, minor groove drug effects on other DNA reactive enzymes have been reported [16–19]. Such inhibition has been correlated with the extent of drug binding to DNA. This report extends the previous study of minor groove agents and topoisomerase I to examine the effects of the three DNA directed drugs shown in Fig. 2 on camptothecin activity. We found that, in intact nuclei, each of these drugs can interfere with the induction of cleavable complex by camptothecin. The results suggest that agents binding to the minor groove of DNA may be useful for manipulating the action of anti-topoisomerase drugs.

MATERIALS AND METHODS

Preparation of nuclei. Logarithmically growing suspension cultures of L1210 were maintained and radiolabeled with [^{14}C]thymidine, and nuclei were prepared as described elsewhere [20]. Briefly, cells washed with phosphate-buffered saline (PBS) were resuspended in nuclei buffer (0.002 M potassium phosphate, pH 6.9, 0.005 M $MgCl_2$, 0.150 M NaCl, 0.001 M EDTA) and centrifuged at 100 *g* for 4 min. The cell pellet was resuspended in nuclei buffer at 3×10^6 cells/mL and adjusted to 0.3% (v/v) Triton X-100. After 20 min at 4°, samples were centrifuged at 300 *g* for 13 min, and the pellets were resuspended in nuclei buffer at 10^6 nuclei/mL.

Drug incubations and determination of DNA–protein crosslinks and single-strand breaks. Drugs were added as indicated to 1-mL aliquots of nuclei at 4° followed by incubation for 5 min at 37°. Reactions were stopped by rapid cooling to 4°. Detection of DNA–protein crosslinks was performed as described elsewhere [21, 22]. Briefly, samples were diluted 1:1 (v/v) with hot (65°) lysis buffer [3% sodium dodecyl sulfate (SDS), 40 mM EDTA, pH 8.0, 400 μg/mL salmon testes DNA), heated for 15 min at 65° and sheared using a Vortex-Genie (Scientific Industries) at high speed for 30 sec. Samples were adjusted to 65 mM potassium chloride and placed at 4° for 15 min. After centrifugation at 4° for 15 min at 800 *g*, the precipitated DNA was resuspended in wash buffer (10 mM Tris–HCl, pH 8.0, 1.0 mM EDTA, 100 mM potassium chloride, and 100 μg/mL salmon testes DNA). Samples were heated at 65° for 5 min, then returned to 4° for 15 min and centrifuged as above. After two more cycles of resuspension and centrifugation, precipitates were dissolved in 2 M perchloric acid and radioactivity was determined by liquid scintillation counting. Single-strand breaks were monitored by alkaline elution as follows. After drug treatment, samples were placed at 4° and 5×10^5 nuclei applied to a polyvinyl chloride (0.2 mM) filter preequilibrated with cold (4°) PBS. Samples were washed twice with 10 mL of cold PBS and then incubated for 30 min with 250 μg/mL proteinase K in 2% SDS, 0.25 M tetrasodium EDTA, pH 9.7. Samples were eluted with 30 mL of 2% tetrapropylammonium hydroxide, pH 12.1, 20 mM EDTA. Total elution time was 1 hr. Radioactivity in the fractions was determined by liquid scintillation counting. The theory and application of alkaline elution have been detailed elsewhere by Kohn and coworkers [23, 24].

Reagents and chemicals. Distamycin A, DAPI, proteinase K, salmon testes DNA, dithiothreitol, bovine serum albumin, Triton X-100, and agarose were purchased from the Sigma Chemical Co. (St Louis, MO). Hoechst 33258 was obtained from the Aldrich Chemical Co. (Milwaukee, WI). Distamycin, DAPI and Hoechst were dissolved in water at 1 mM concentrations and stored at −20°. Camptothecin was obtained from NCI and dissolved at 10 mM in dimethyl sulfoxide. All other chemicals used were reagent grade.

RESULTS

Generation of camptothecin-induced "cleavable complex" is characterized by covalent DNA–protein crosslinks and single-strand breaks [8]. These lesions reflect the presence of transient DNA intermediates formed during strand passage by topoisomerase I. Agents which interfere with camptothecin activity would be exptected to limit formation of these lesions. Since minor groove binding drugs were shown earlier to alter the catalytic activity of topoisomerase I [14], it was of intererest to determine whether modulation of camptothecin activity was similarly affected.

The effect of distamycin on nuclear DNA–protein crosslinks induced by camptothecin is shown in Fig. 3. Camptothecin alone (0.5 to 50 μM) effected crosslinking of 12–56% of nuclear DNA (Fig. 3a). A limited increase in crosslinks was noted between 5

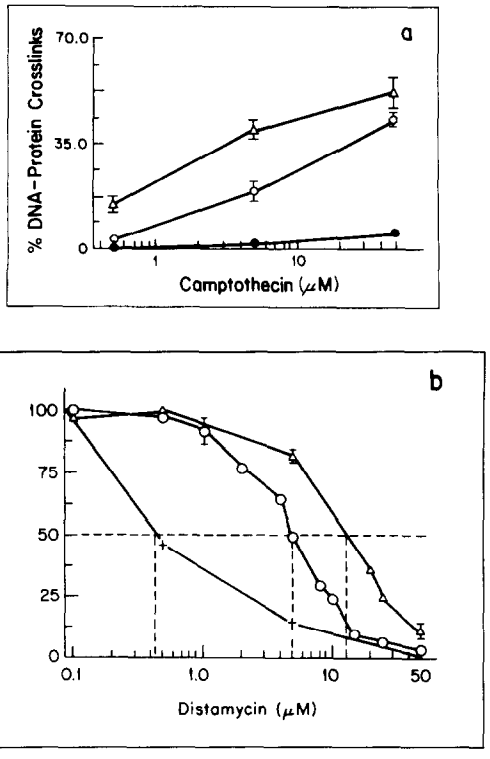

Fig. 3. Effect of distamycin on DNA–protein crosslinks induced by camptothecin. [^{14}C]Thymidine-labeled L1210 nuclei were prepared as described in Materials and Methods and incubated with 0.5, 5 or 50 μM camptothecin in the presence or absence of distamycin. After 5 min at 37°, DNA–protein crosslinks were determined by potassium–sodium lauryl sulfate precipitation as described in Materials and Methods. Data points are means ± SE and represent four independent experiments. (a) camptothecin alone (△—△); camptothecin plus 5 μM distamycin (○—○); and camptothecin plus 50 μM distamycin (●—●). (b) The data have been normalized such that 100% equals the amount of DNA–protein crosslinks observed at any one dose of camptothecin in the absence of distamycin. Three camptothecin levels are shown: 0.5 μM (+—+); 5 μM (○—○); and 50 μM (△—△), which in the absence of distamycin effected crosslinking of 52, 40, and 15% of the DNA respectively.

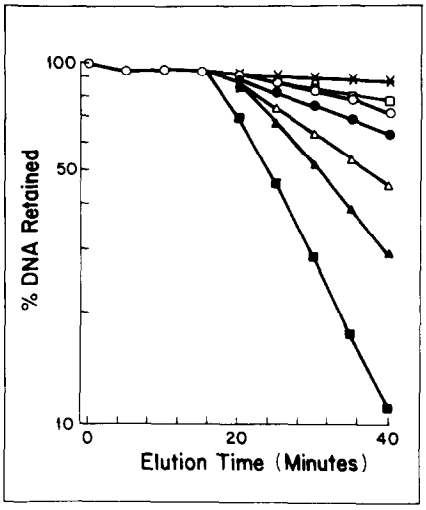

Fig. 4. Distamycin inhibition of DNA single-strand breaks induced by camptothecin. Nuclei prepared as described in Fig. 3 were incubated with camptothecin in the absence or presence of equimolar concentrations of distamycin. After 5 min at 37°, nuclei were subjected to alkaline elution as described in Materials and Methods. Key: (■—■) 50 μM camptothecin; (□—□) 50 μM camptothecin, 50 μM distamycin; (▲—▲) 5 μM camptothecin; (△—△) 5 μM camptothecin, 5 μM distamycin; (●—●) 0.5 μM camptothecin; (○—○) 0.5 μM camptothecin, 0.5 μM distamycin; and (×—×) control (no drug).

and 50 μM camptothecin. DNA–protein crosslinks were not produced by distamycin treatment alone, in the absence of camptothecin (data not shown). However, addition of 5 or 50 μM distamycin to the camptothecin–nuclei reaction reduced lesion formation significantly, resulting in nearly complete inhibition of crosslinks by 50 μM distamycin. In Fig. 3b, the data were normalized to allow direct comparison of distamycin effects at three concentrations of camptothecin. At low camptothecin concentrations, distamycin inhibited crosslink formation on a mole/mole (distamycin/camptothecin) basis. Crosslinks induced by 0.5 and 5 μM camptothecin decreased by 50% in the presence of comparable concentrations of distamycin (0.45 and 5 μM respectively). However, only 12 μM distamycin was needed to reduce by 50% crosslinks induced by 50 μM camptothecin. This observation may be explained by the data shown in Fig. 3a. Absolute amounts of crosslinked DNA produced by 50 μM camptothecin alone were increased only 23% over that observed with 5 μM drug, suggesting some degree of saturation of camptothecin reaction sites. If such saturation had indeed occurred, the amount of distamycin required to inhibit the reaction might also be reduced.

The ability of minor grove ligands to interfere with camptothecin-induced DNA single-strand breaks was also examined. Figure 4 shows a typical DNA alkaline elution profile obtained when nuclei were reacted with camptothecin in the absence or presence of equimolar distamycin concentration. Upon alkaline elution, 63, 29, or 11% of DNA was retained on the filters when nuclei were treated with 0.5, 5 or 50 μM camptothecin alone, respectively. Breakage was reduced when distamycin was added simultaneously with camptothecin. At 0.5 and 5 μM distamycin, strand breakage induced by equimolar levels of camptothecin decreased by 25–30%. At higher levels of camptothecin and distamycin, inhibition was even more pronounced. At 50 μM, distamycin reduced camptothecin damage by 87%. Additionally, no strand breaks were observed when nuclei were treated with distamycin in the absence of camptothecin (data not shown). Thus, camptothecin-mediated formation of both crosslinks and strand breaks was reduced by distamycin.

The inhibition of lesion formation described above was observed with simultaneous addition of distamycin and camptothecin to nuclei. Whether distamycin could reverse camptothecin damage, in addition to preventing it, was determined by changing the order of drug addition (Fig. 5). When drugs

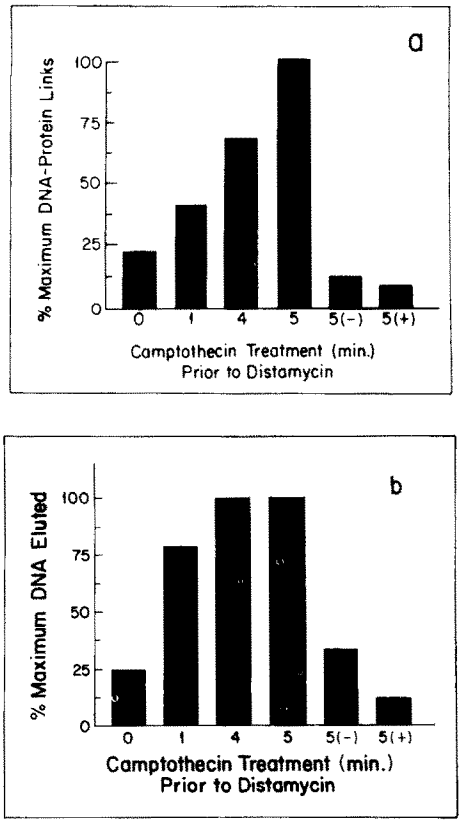

Fig. 5. Effect of time of addition of distamycin on camptothecin-induced DNA lesions. Nuclei prepared as in Fig. 3 were preincubated at 37° with 5 μM camptothecin for the times shown before addition of 10 μM distamycin. Total incubation time with camptothecin was 5 min. Samples were analyzed for the presence of DNA–protein crosslinks (a) and single-strand breaks (b) as described in Materials and Methods. 5(−) and 5(+): At the end of the 5-min incubation, aliquots of nuclei were removed and adjusted to 0 [5(−)] or 10 μM distamycin [5(+)], pelleted by centrifugation at 500 g for 5 min, resuspended in nuclei buffer containing 0 [5(−)] or 10 μM distamycin [5(+)] and further incubated for 30 min at 37°.

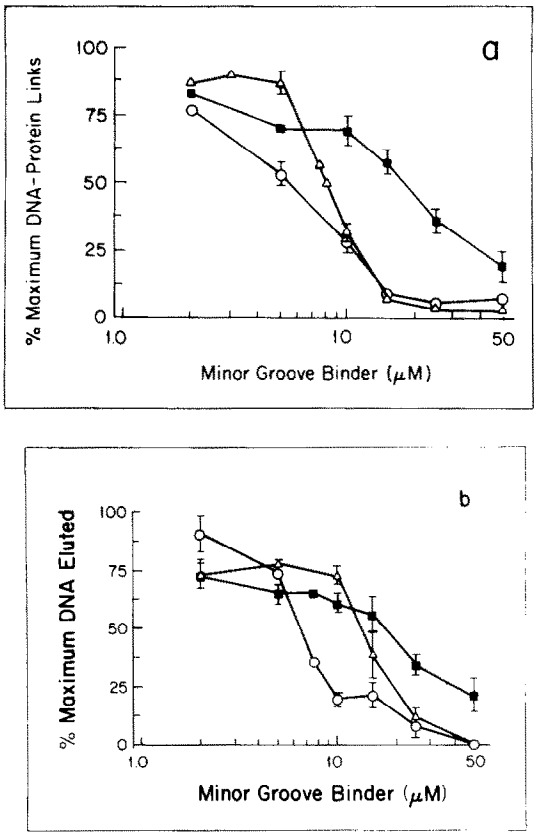

Fig. 6. Comparative effects of minor groove binders on camptothecin-induced DNA lesions. Nuclei prepared as described in Fig. 3 were incubated for 5 min at 37° with 5 μM camptothecin plus increasing concentrations of distamycin (○—○), Hoechst 33258 (△—△) or DAPI (■—■). Samples from five separate experiments were analyzed for the presence of DNA–protein crosslinks (a) and single-strand breaks (b) as described in Materials and Methods. Data are means ± SE.

were added simultaneously (at zero time), camptothecin-induced crosslinks (Fig. 5a) and breaks (Fig. 5b) decrease 80 and 75% respectively. If nuclei were pretreated with 5 μM camptothecin for 1 or 4 min, the effect of distamycin was reduced. With 5 min of camptothecin treatment prior to distamycin addition, DNA damage (both DNA–protein crosslinks and strand breakage) was maximal. Distamycin added 5 min after camptothecin had no effect on either parameter. Thus, inhibition of lesion formation was dependent upon early addition of distamycin. These results reflect the time course of camptothecin-induced crosslinks and breaks in nuclei (data not shown) and indicate that distamycin can inhibit but cannot reverse formation of camptothecin-induced lesions in nuclei.

Repair of camptothecin damage was also monitored. Typically, removal of camptothecin from treated cells results in rapid dissociation of the complex and repair of DNA lesions [9]. DNA binding drugs which inhibit formation of the complex might also limit its dissociation. To examine this possibility, nuclei were treated for 5 min with camptothecin to achieve maximum DNA–protein crosslinks and strand breaks. At this point, the sample was divided in two aliquots to which either 10 μM distamycin or an equivalent volume of water was added. The samples were centrifuged and resuspended in medium containing distamycin or water as above. Crosslinks and strand breaks were determined after 30 min of repair. Distamycin did not prevent repair of either lesion. Nuclear damage was repaired at least as well in the presence [Fig. 5, 5(+): 90%], as in the absence [Fig. 5, 5(−): 70–90%], of 10 μM distamycin. Thus, distamycin inhibited association but not dissociation of the camptothecin "cleavable complex."

It was of interest to determine whether the above observations were unique to distamycin or applicable to other minor groove binding agents. Therefore, DAPI and Hoechst 33258, differing both in molecular weight and in the size of their nucleotide binding sites, were assayed for their effects on camptothecin-induced DNA lesions (Fig. 6). In the

Table 1. Comparative effects of minor groove binders on camptothecin-induced lesions in nuclei*

DNA lesion	Drug required for 50% reducion of camptothecin effect		
	Distamycin (μM)	Hoechst 33258 (μM)	DAPI (μM)
DNA–protein crosslinks	5 ± 0.1	8 ± 0.2	18 ± 1.5
Single-strand breaks	6 ± 0.5	12 ± 0.9	17 ± 2

* Nuclei were incubated with drugs (5 μM camptothecin plus various concentrations of single minor groove binders). After 5 min at 37°, DNA–protein crosslinks were assayed by potassium–sodium lauryl sulfate precipitation and single-strand breaks by alkaline elution. With 5 μM camptothecin alone, 40% of DNA was crosslinked with protein, while 30% was retained on the filter after alkaline elution. The data presented resulted from five independent experiments and are expressed as means ± SE.

absence of camptothecin neither agent produced crosslinks or strand breaks (data not shown). Yet, both Hoechst 33258 and DAPI inhibited camptothecin-induced lesions. At the lowest drug levels (2 and 5 μM) Hoechst 33258 reduced crosslink formation to a lesser extent than either DAPI or distamycin (Fig. 6a). Above 5 μM however, Hoechst-treated samples exhibited a sharp reduction in crosslinks such that by 10 μM these samples were equivalent to distamycin-treated nuclei. At higher drug concentrations, DAPI was less effective than either distamycin or Hoechst. Less than 10% of camptothecin-treated nuclear DNA was linked to protein in the presence of 15 μM distamycin or Hoechst compared to 55% with DAPI. When the same samples were subjected to alkaline elution, reduction of camptothecin-induced strand breaks was also observed (Fig. 6b). Between 5 and 15 μM, distamycin was more potent than either DAPI or Hoechst in reducing strand breakage. With 10 μM minor groove agent, only 20% of camptothecin-induced strand breaks persisted in the presence of distamycin, compared to 60% with DAPI and 70% with Hoechst. Above 10 μM, Hoechst caused a sharp reduction in strand breaks compared to a more gradual decline observed with DAPI. By 25 μM, Hoechst effects were equivalent to distamycin. At this level of distamycin, camptothecin-induced strand breakage was only 10% of that occurring in the absence of minor groove binder. By contrast, 30% of this damage was still observed in nuclei treated with 25 μM DAPI. A summary of drug effects on formation of both crosslinks and strand breaks is presented in Table 1. In this study, drug levels required to effect a 50% reduction in camptothecin damage were defined. The order of inhibitory potency for both lesions was distamycin > Hoechst 33258 > DAPI. Reduction in crosslinks closely paralleled reduction in breaks with both distamycin and DAPI, whereas slightly higher levels of Hoechst 33258 were required for inhibition of strand breaks than crosslinks. Distamycin was 3-fold more potent than DAPI in inhibiting formation of both lesions, with Hoechst showing intermediate activity. Thus, despite differences in effective concentration, all three ligands were capable of inhibiting nuclear camptothecin damage.

In summary, distamycin inhibition of camptothecin-induced crosslinks and strand breaks was dependent on time of addition and drug concentration. Decreased association rather than altered dissociation of the complex was observed. Production of camptothecin damage was also inhibited by two other minor groove binders, DAPI and Hoechst 33258. None of the minor groove binders produced crosslinks or strand breaks in the absence of camptothecin.

DISCUSSION

The DNA minor groove ligands distamycin, Hoechst 33258 and DAPI were shown previously to alter topoisomerase I-mediated relaxation of DNA [14]. In the current report, these same agents decreased the activity of the topoisomerase I-specific inhibitor, camptothecin. DNA–protein crosslinks and strand breaks are associated with camptothecin activity and probably result from formation of a camptothecin–topoisomerase I–DNA "cleavable complex" [8]. Distamycin A reduced these lesions in a time- and concentration-dependent manner by preventing lesion formation.

Minor groove ligands reportedly effect numerous cellular changes. Elongation of the G_2 phase [25], decreased chromatin compaction [25, 26], reduced DNA and RNA synthesis [19, 25, 27], and chromosome breakage [28, 29] are attributed to the ability of these drugs to bind cellular DNA. Distamycin, Hoechst 33258 and DAPI are classical minor groove binding agents with K_a values (for calf thymus DNA) ranging from $1.0 \times 10^6\ M^{-1}$ (distamycin and Hoechst 33258) to $5 \times 10^6\ M^{-1}$ (DAPI) [30–32]. As shown in Fig. 2, these agents possess a crescent shaped structure which presumably facilitates their fit into the minor groove of DNA [33, 34]. Despite their similarities, these agents are not identical in action. In general, preferential binding to runs of A–T base pair sequences is observed [15, 19]. However, Hoechst 33258 and distamycin tolerate G-C pairs more readily than DAPI [35]. Indeed, Hoechst may require a G-C sequence for binding of its piperazine ring [33].

In the current study, while all three agents inhibited cleavable complex, some differences were noted. For example, crosslinks were somewhat more sensitive to Hoechst 33258 inhibition than were strand breaks. By contrast, crosslink reduction more closely paralleled strand break inhibition with distamycin and DAPI. Whether the reported differences among these drugs in nucleotide specificity might account for this discrepancy is uncertain. These agents also differ in the size of their nucleotide binding site, distamycin requiring 5 base pairs, compared to 3 for DAPI and 4–5 for Hoechst. Finally, the size of the drug molecules differs. The order of potency reported herein for nuclear camptothecin inhibition correlated both with the size of the drug molecules and their nucleotide binding sites: distamycin > Hoechst 33258 > DAPI. Notably, drug potency did not correspond to reported variations

in drug–DNA binding constants. However, these constants reflect an affinity for purified DNA rather than for nuclear chromatin, the drug target in the present study.

Interestingly, no crosslinks or strand breaks were observed when nuclei were treated with distamycin, DAPI or Hoechst in the absence of camptothecin. Thus, "camptothecin-like" inhibition of topoisomerase I (i.e. via "cleavable complex" formation) does not appear to be a property of these agents. However, this does not preclude inhibition of the enzyme based on decreased association with its target, DNA, or reduced turnover of the enzyme–DNA complex. Indeed, in our earlier work direct inhibition of L1210 topoisomerase I catalysis with distamycin, Hoechst 33258 and DAPI was reported [14]. The order of drug potency for inhibition of isolated topoisomerase I reported therein was similar to that observed here in the nuclear studies. Additionally, these same minor groove drugs induced alterations in both the catalytic and decatenation activities of isolated L1210 topoisomerase II [36]. Nuclear studies showing similar effects on VM-26 (teniposide) and *m*-AMSA [4′-(9-acridinylamino)methane - sulfon - *m* - anisidide] induced topoisomerase II-mediated DNA lesions have also been reported [20]. In view of the diversity of these systems involving both topoisomerase I- and topoisomerase II-mediated activities, drug interaction with DNA is a likely explanation for the similarity of the minor groove drug effects.

The complexity of the camptothecin–topoisomerase I–DNA interaction suggests a number of possible mechanisms for alteration by minor groove drug agents. Camptothecin, by binding to the topoisomerase I–DNA complex, may effect a shift in equilibrium from the non-cleavable to the cleavable complex [8]. Interference with this activity by DNA ligands could result from an inhibition of topoisomerase I–DNA binding, decreased association of camptothecin with the topoisomerase I–DNA complex, or alterations in the camptothecin–topoisomerase I–DNA complex. Decreased enzyme–DNA binding may well be affected by drug masking of specific nucleotide recognition sequences. Binding of topoisomerase I to DNA reportedly occurs at a specific hexadecameric nucleotide sequence in which the presence of a core thymidine is crucial to enzyme activity [12, 37]. Since minor groove ligands bind preferentially to runs of A-T base pairs [15], such binding could effectively mask the recognition sequence and reduce topoisomerase I–DNA association.

Minor groove drugs may also reduce association of camptothecin with the topoisomerase I–DNA complex. While stabilization of cleavable complex presumably results from camptothecin binding to topoisomerase I, the possibility remains that once bound, camptothecin may itself interact with DNA. Such an interaction could be affected by drugs binding to the DNA minor groove.

Lastly, DNA minor groove binding drugs may inhibit camptothecin activity by altering the "cleavable complex." Certain minor groove drugs reportedly stabilize DNA structure, and both reduced unwinding of duplex DNA strands [25] and stabilization of the B form of the helix [17] have been described. Such stabilization may interfere with the DNA swiveling associated with cleavable complex formation [8, 38]. The data presented in this study are consistent with drug inhibition by any of these mechanisms (i.e. altered enzyme–DNA binding, reduced camptothecin–topoisomerase I association or alterations in cleavable complex). However, our previous study showing minor groove drug effects on isolated L1210 topoisomerase I in the absence of camptothecin suggests that an alteration in topoisomerase I–DNA binding is likely. That the order of drug potency is the same in both nuclear and enzyme studies lends credence to the idea that the topoisomerase I–DNA interaction is being affected in both systems.

Despite their ability to reduce camptothecin complex formation, minor groove agents did not promote or inhibit removal of performed lesions. Two reasons for such ineffectiveness may be postulated: (1) the DNA orientation required for the camptothecin–enzyme–DNA complex cannot be altered once the complex has formed; or (2) DNA associated with the complex is no longer accessible to distamycin binding. The data presented here cannot distinguish between these two possibilities. However, analogous effects of distamycin on *Escherichia coli* RNA polymerase have been reported by Straney and Crothers [17]. These authors suggested that accessibility of the DNA to drug binding was reduced in the initiated compared to the open complex. A similar reduction of DNA accessibility to distamycin may result from camptothecin complex formation. Direct competition by camptothecin and distamycin for DNA binding sites is not a factor since camptothecin has never been shown to bind directly to DNA [3].

Topoisomerase activity is known to be affected by a number of DNA reactive agents [39]. DNA intercalators may inhibit enzyme activity because of their ability to unwind DNA or by some as yet undefined mechanism [13, 40]. While reports of inhibition by Adriamycin® are limited to topoisomerase II [37, 41], the activities of both topoisomerases are affected by ellipticine and the 9-aminoacridines [13, 42, 43]. In addition, intercalation can inhibit epipodophyllotoxin-induced stabilization of topoisomerase II cleavable complex [44]. Such inhibition also has been reported for agents which induce changes in DNA conformation without binding to the helix. Inhibitors of polyamine biosynthesis reportedly affect *m*-AMSA inhibition of topoisomerase II by this mechanism [45]. However, despite the availability of studies detailing alterations in topoisomerase II cleavable complex stabilization, the current study is the first example of comparable drug effects on the topoisomerase I induced complex.

Modulation of camptothecin is important in view of the chemotherapeutic activity of the drug. Such modulation may be effected by localized changes in helical structure. Minor groove drugs are known to cause limited changes in the narrow groove coincident with or localized to the drug binding site [15]. The present work demonstrates the abilities of these drugs to inhibit camptothecin reactivity. Determining the precise DNA modification responsible

for minor groove drugs effects on camptothecin will require further analysis. Yet, it is apparent that these drugs provide a novel approach for study of DNA drug-induced modulation of topoisomerase I-mediated cleavable complex.

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