0026-895X/93/051064-06$03.00/0

MOLECULAR PHARMACOLOGY, 44:1064-1069

Antihelicase Action of DNA-Binding Anticancer Agents: Relationship to Guanosine-Cytidine Intercalator Binding

NICHOLAS R. BACHUR, ROBIN JOHNSON, FANG YU, ROBERT HICKEY, NANCY APPLEGREN, and LINDA MALKAS

Departments of Medicine (N.R.B.), Pharmacology and Experimental Therapeutics (N.R.B., F.Y., R.H., N.A., L.M.), and Program of Oncology (N.R.B., R.J., F.Y., R.H., L.M.), University of Maryland School of Medicine, and Department of Pharmaceutical Sciences (R.H.), University of Maryland School of Pharmacy, Baltimore, Maryland 21201

Received May 5, 1993; Accepted September 2, 1993

SUMMARY

DNA-binding antibiotics such as intercalators, narrow groove binders, and other substances modify duplex DNA, making it an altered substrate for DNA helicases. The intercalators daunorubicin, actinomycin D, echinomycin, and elsamicin, the narrow groove binders distamycin and mithramycin, and the plant toxin teniposide, each representing a different chemical class, block SV40 large T antigen DNA helicase action with IC_{50} values ranging from 4×10^{-8} to 2×10^{-6} M. A partially purified human HeLa cell DNA helicase is also potently blocked by daunorubicin, distamycin, and teniposide. Because eukaryotic cells contain helicases of varying abundance, specificity, and type, this site of action for DNA-binding antibiotics may help explain antibiotic potency and specificity for DNA or RNA inhibition. The antihelicase effect of the antibiotic-double-stranded DNA complex may be central to the anticancer activities of these substances. An additional interesting correlation is the antihelicase action of DNA-intercalating antibiotics and their DNA-binding preference for G-C base pair sites. The G-C base pair binding preference of the intercalating antibiotics may result from evolutionary selection because of the higher G-C binding stability, compared with A-T binding stability. The combination of the higher base pair stability at G-C regions and increased duplex DNA stability induced by intercalating antibiotic yields a total additive stability of the intercalator-G-C base pair complex that resists helicase action.

DNA-binding antibiotics comprise a substantial proportion of the clinically useful anticancer drugs. Most of these DNA-binding antibiotics complex preferentially with double-stranded (duplex) DNA (1). Several biochemical consequences of this interaction between these antibiotics and duplex DNA have been described and range from the inhibition of DNA synthesis, RNA synthesis, and DNA repair to more recently described inhibition of topoisomerases (2). Although structural mechanisms for the binding of some of these antibiotics to duplex DNA have been extensively characterized, the subsequent biochemical events that result from the physical binding are controversial and not fully understood.

Duplex DNA must be converted to single-stranded DNA for biochemical and molecular processing of the DNA base sequence information. A family of enzymes, the DNA helicases, dissociate duplex DNA to provide single-stranded DNA template, which is essential for the replication and transcription processes (3).

Anthracyclines and other DNA-intercalating drugs increase the melting temperature and, therefore, increase the stability of the interstrand binding of double-stranded DNA (1). Therefore, we postulated that the intercalated anthracyclines would interfere with the enzymatic separation of the paired DNA double strands (4). In our investigations of this hypothesis, we found that anthracycline antibiotics bind to double-stranded DNA and potently block viral and eukaryotic DNA helicase action on the double-stranded DNA. From these studies, we presume that the intercalating anthracyclines bind and modify the duplex DNA, resulting in an antibiotic-duplex DNA complex that functions poorly as a helicase substrate.

Our evaluation of the relationship between the potency of the helicase blockade by a series of anthracyclines (micromolar IC_{50}) and the increase in stability of DNA interstrand binding produced by these anthracyclines (ΔT_m) indicated that these two factors may be related but that other factors must also contribute to the antihelicase action (4). We also suggest that a DNA-binding drug may show preference for inhibition of DNA or RNA synthesis, depending on the type of helicase that is principally affected by the drug-DNA complex. Although we have established the antihelicase activities of the anthracycline antibiotics with virally induced, human, and murine helicases, we must yet evaluate DNA- and RNA-specific helicases to determine preferential drug activities.

In addition to our studies with eukaryotic DNA helicases, other studies have recently reported on the activities of prokaryotic DNA helicases and CC1065 and its analogs (5, 6) and of several DNA-intercalating compounds (7).

We have expanded our investigation of the antihelicase activity of DNA-binding antibiotic substances to include several other classes of DNA intercalators, narrow groove binders, and

This research was supported by a University of Maryland Bressler Award to N.R.B., American Cancer Society Maryland Division Awards to R.H. and L.M., Leukemia Research Foundation Award to R.H., and National Leukemia Association Award to L.M.

other types of anticancer drugs. From our observations, we also propose an explanation for the general G-C base pair binding preference of DNA-intercalating antibiotics.

Materials and Methods

The DNA-binding agents we tested (Fig. 1) were obtained from several sources. Actinomycin D and etoposide were obtained from Sigma Chemical Co. Daunorubicin, distamycin, echinomycin, and teniposide were obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, National Cancer Institute. Elsamicin was a gift of Bristol-Myers Squibb Corporation. M13Mp19 (+) circular DNA and the 17-mer 5′-TCATGGTCATAGCTGTT were purchased from GIBCO, Bethesda Research Laboratories. Other chemicals and reagents were the highest grade commercially available, and solutions were made in ultrapure deionized water except where noted otherwise.

HeLa cells and murine mammary carcinoma FM-3A cells were grown in culture in Joklik's modified Eagle medium supplemented with 5%

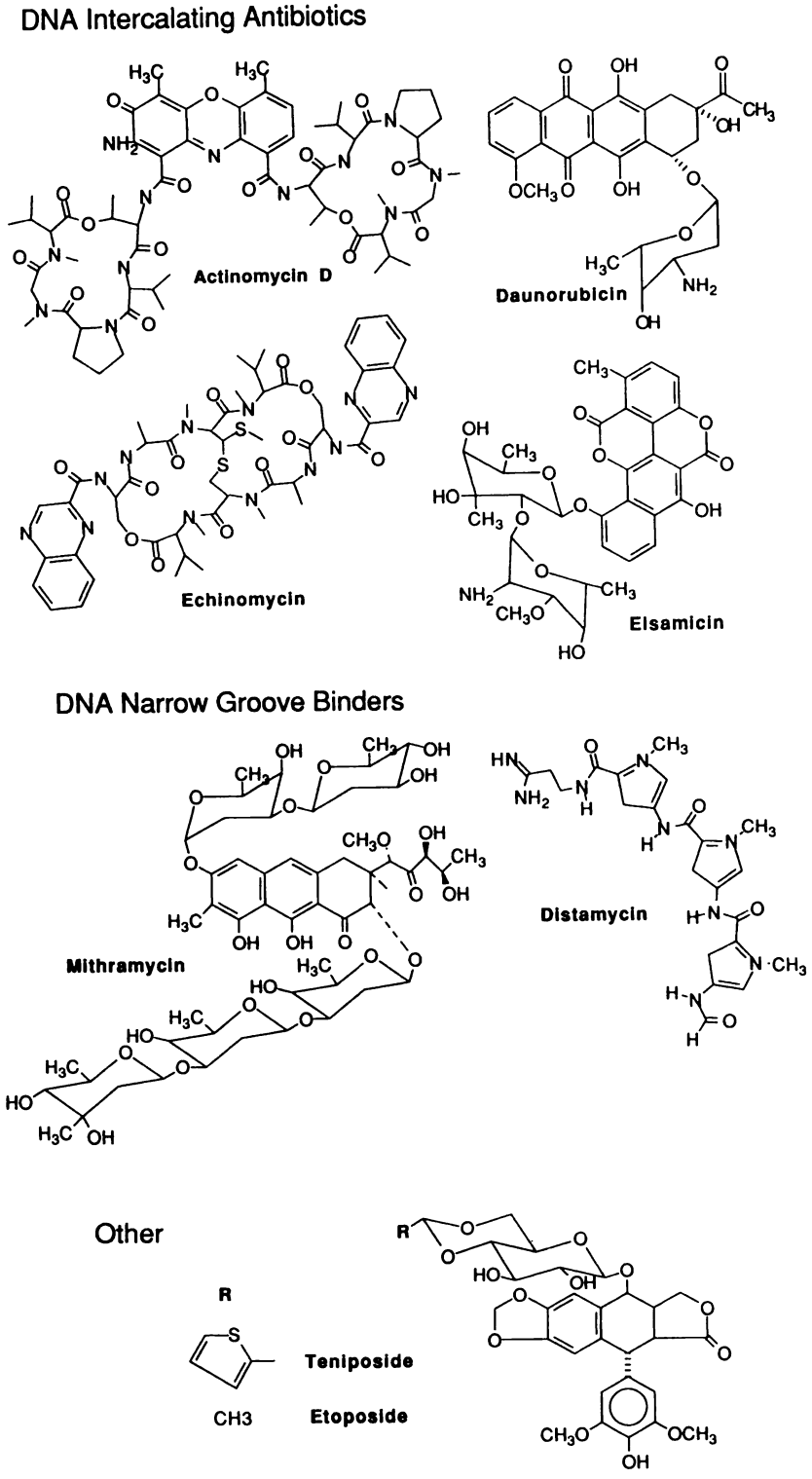

Fig. 1. Structural formulae of DNA-binding anticancer agents assessed for antihelicase activity.

each of irradiated calf and fetal bovine serum. The SV40 large T antigen was purified from CV-1 cells infected with helper-dependent recombinant adenovirus vector Ad-SVR 284 by a modification of the immunoaffinity procedure of Simanis and Lane (8), as described previously (4). Helicases from HeLa cells and FM-3A cells were isolated according to a modified method of Malkas *et al.* (9). Helicase substrate preparations and the helicase assay were as described previously (4).

The antibiotic stock solutions were made in dimethylsulfoxide and were stored at −20°. For the enzymatic assays, the antibiotics were diluted in 10 mM Tris·HCl, pH 7.4, before mixing with the helicase M13–17-mer DNA substrate. Dimethylsulfoxide was tested at concentrations up to 0.028 M and did not affect control helicase reactions.

Results

We tested and compared representative examples of DNA-intercalating antibiotics of four separate antibiotic classes, i.e., actinomycins, anthracyclines, chartarins, and quinomycins, for their effects on SV40 large T antigen helicase action. Although these intercalators represent different chemical structural families, they all contain a combination of planar ring systems and peptide or glycoside moieties (Fig. 1). These chemical structures contribute several functional characteristics to all the intercalating antibiotics. The planar ring systems provide hydrophobic intercalating plates, and the peptide or glycoside provides hydrophilic polarity as well as narrow groove-binding and -stabilizing characteristics. Another important similarity among these intercalating antibiotics is their DNA-binding preference for G-C base pairs. Daunorubicin (10), actinomycin D (11), and elsamicin (12) bind preferentially to GpC sequences in duplex DNA, whereas echinomycin (13) binds preferentially to CpG sequences. However, these studies show that these antibiotics also may bind to other G/C-containing sequences, such as GpG, with lower affinities. Each of these DNA-intercalating agents displayed high potency against the SV40 large T antigen DNA helicase activity (Table 1). Actinomycin D blocked T antigen helicase with an IC_{50} of 0.8 μM. The anthracycline antibiotic daunorubicin had an antihelicase action IC_{50} of 0.4 μM (Fig. 2; Table 1). Elsamicin, a chartarin-based antibiotic, potently interfered with helicase activity, with an IC_{50} of 0.15 μM. Echinomycin, a quinomycin bis-intercalator with a large peptidyl moiety that occupies the minor groove, was the most potent blocker of T antigen helicase, with an IC_{50} of 0.04 μM. We did not see a loss of inhibitory activity at higher concentrations of these intercalators, as has been described for topoisomerase II inhibition (14, 15).

Distamycin and mithramycin represent antibiotics of two different chemical classes that bind in the narrow grove of duplex DNA (16, 17) (Fig. 1). The T antigen helicase blockade IC_{50} values shown by distamycin and mithramycin were 2 μM and 1 μM, respectively (Fig. 2; Table 1). This helicase blockade by the narrow groove binders was concentration dependent and similar to the blockade by intercalators.

Another class of DNA-modifying substances, the epipodophyllotoxins etoposide and teniposide, are potent topoisomerase II inhibitors that bind to the topoisomerase proteins and were recently shown to bind to DNA (18) (Fig. 1). Teniposide blocked T antigen helicase with an IC_{50} value of 0.8 μM (Fig. 2; Table 1). Although this compound is not commonly regarded as a DNA ligand, this inhibitory activity provides evidence that the agent does have DNA-binding capability. Interestingly, the very structurally similar etoposide did not block T antigen helicase significantly.

We also evaluated the sensitivity of human helicase activity from HeLa cells to an intercalator, a narrow groove binder, and the podophyllotoxins (Fig. 3). This human cell helicase activity was less sensitive than the T antigen helicase to these DNA-binding drugs but the blockade by the agents was still quite potent. The IC_{50} of daunorubicin for HeLa helicase was 0.9 μM or about twice that for the T antigen helicase. Similarly, the IC_{50} of distamycin for HeLa helicase was 4.5 μM, again about twice that for the T antigen helicase. Teniposide showed the greatest difference between helicase sensitivities, with an IC_{50} for HeLa helicase of 7.0 μM, which is almost 10-fold the value for the blockade of T antigen helicase (0.8 μM). Etoposide, an analog of teniposide, again had very little activity against the HeLa cell helicase, as with T antigen helicase.

To evaluate possible effects of the anticancer agents on the T antigen helicase protein, we preincubated the agents with the helicase rather than with the duplex DNA substrate. These enzyme preincubation experiments showed no additional effects on the degree of helicase blockade.

Over the course of these experiments, we have conducted many evaluations of control compounds. We found that numerous anticancer drugs that do not bind to DNA effectively also do not affect the helicase action of T antigen helicase. Such anticancer drugs as methotrexate, porfiromycin, cyclophosphamide, and vindescine did not block helicase at concentrations up to 100 μM.

TABLE 1

Antihelicase action of DNA modifiers

IC_{50} values are concentrations of modifiers needed to produce 50% inhibition of the helicase. Values are the average ± standard deviation of three to five experiments.

	IC_{50}	
	T antigen helicase	HeLa cell helicase
	μM	
Reversible intercalators		
Actinomycin D	0.8 ± 0.14	ND[a]
Daunorubicin	0.4 ± 0.14	0.9 ± 0.14
Echinomycin	0.04 ± 0.007	ND
Elsamicin	0.15 ± 0.07	ND
Narrow groove binders		
Distamycin	2 ± 1	4.5 ± 0.7
Mithramycin	1 ± 0.49	ND
Other		
Teniposide	0.8 ± 0.07	7.0 ± 2.0
Etoposide	Not effective	>80

[a] ND, not done.

Discussion

We previously showed that a series of anthracycline antibiotics block virally induced T antigen helicase, human HeLa cell helicase, and murine FM-3A cell helicase activities (4). Now we extend this fundamental mechanism of drug action to include other families of DNA intercalators, DNA narrow groove-binding antibiotics, and the epipodophyllotoxin teniposide. The antihelicase action of these disparate types of antibiotics may be central to the anticancer activities of these agents.

Although the increased stability of the intercalated antibiotic-duplex DNA complex alone may account for the blockade of helicase action, both intercalating antibiotics and the narrow groove binders modify duplex DNA in other ways that also may

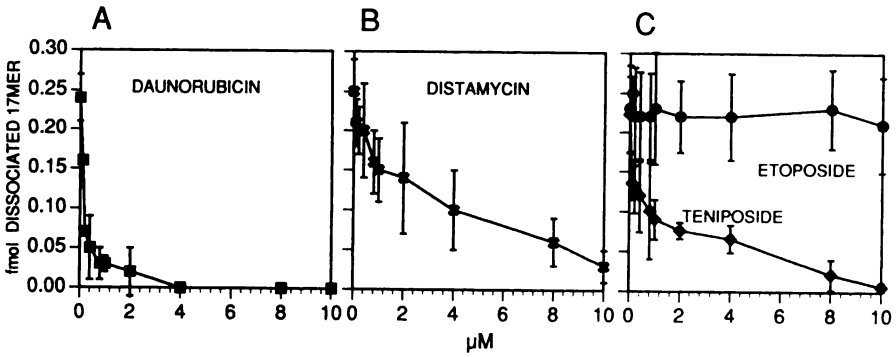

Fig. 2. Blockade of T antigen helicase activity by DNA-binding anticancer agents. DNA helicase substrate was single-stranded phage M13 DNA annealed to a complementary ^{32}P-labeled 17-mer (5′-TCATGGTCATAGCTGTT). A, Daunorubicin; B, distamycin; C, teniposide and etoposide. The *plots* show the means of three to five experiments, with standard deviations shown.

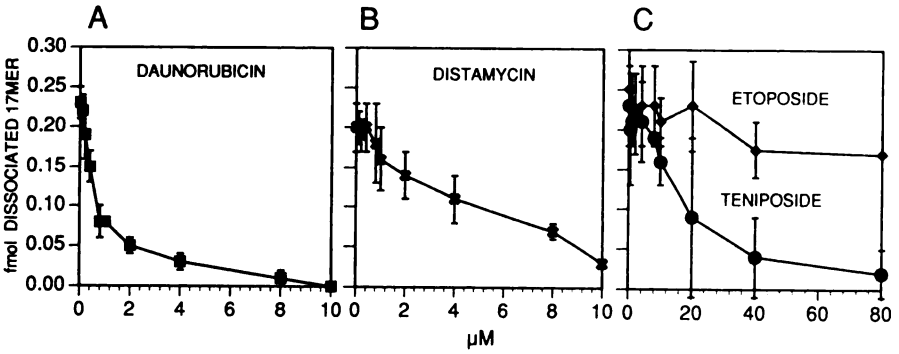

Fig. 3. Blockade of purified HeLa DNA helicase activity by DNA-binding anticancer agents. A, Daunorubicin; B, distamycin; C, teniposide and etoposide. The *plots* show the means of three to five experiments, with standard deviations shown.

be detrimental to helicase action. The structural and topological characteristics of DNA are modified by these ligands. The ligands may increase the DNA helix rigidity and distort, lengthen, and unwind the DNA helix (1). These induced distortions of the helical duplex DNA may reduce its suitability as a helicase substrate.

Another major consideration of the antihelicase action is the space-occupying characteristic of the ligands. For example, structural groups of the intercalating antibiotics that may contribute to the stabilization of intercalative binding, such as the sugars of anthracycline (19) or the peptides of actinomycin D (20), protrude into the narrow groove of the distorted DNA helix and may interfere sterically with helicase movement and function. Narrow groove binders may not stabilize the base pair binding of duplex DNA as effectively as intercalators, but their space occupation in the narrow groove may be important for obstructing the processive movement of some helicases on the DNA strands.

Both the intercalating and the narrow groove-binding antibiotics have base sequence specificity for DNA binding, which must affect their inhibitory potency with our 17-mer-M13 duplex. Our standard DNA substrate, 5′-TCATGGTCATAGCTGTT, contains one GpC binding site and one GpG site, which may offer preferred intercalative binding. Intercalator molecules may bind to these sites preferentially at low drug concentrations, with cooperative binding of additional drug molecules into other available positions until the 17-mer is saturated with several intercalated drug molecules at higher drug concentrations (21).

The narrow groove binders have specific binding sites available in the 17-mer-M13 duplex, such as the GpC and GpG pairs for mithramycin (17, 22) and an ApTpA sequence for distamycin (23). Although distamycin has shown preference for longer A/T-rich binding sequences (16, 23), substantial blockade occurs with our 17-mer, containing only the 3-base pair ApTpA. Therefore, we presume that the 3-base pair ApTpA region provides sufficient binding characteristics for distamycin interaction with our 17-mer-M13 duplex substrate. This shortened binding region for distamycin may account for the somewhat less effective antihelicase action of this narrow groove-binding antibiotic, compared with the intercalators. Further experimentation with A/T-rich binding regions of other lengths can provide additional data on distamycin action, potency, and specificity.

One possible reason for the different antihelicase effects of the structurally similar teniposide and etoposide is DNA base sequence preference for binding. Although no definitive DNA-binding mechanism is known for the epipodophyllotoxins, there may be differential binding in our 17-mer duplex model that results in the different antihelicase activities.

DNA-binding antibiotics inhibit both DNA and RNA synthesis, but to different degrees (24–27). A mechanism for such preferential inhibition could reside at the level of helicase blockade. Because different specific DNA helicases participate during DNA replication and transcription (3), an antibiotic bound to double-stranded DNA may block one type of helicase more than another. This selective blockade would result in preferential inhibition of DNA or RNA synthesis through preferential blockade of helicase activities, even though the antibiotics bind to duplex DNA as their primary mechanism. Although both echinomycin and actinomycin D are more potent inhibitors of RNA synthesis than DNA synthesis (26, 27), their degrees of inhibition of our model T antigen helicase are not equivalent to their published RNA-inhibitory activities. The mechanism for preferential DNA or RNA inhibition remains to be tested, by comparing replication- and transcription-specific DNA helicases for their sensitivities to these DNA-binding antibiotics.

Unlike *Escherichia coli* helicase II, which is reported to be insensitive to actinomycin D (7), we find that T antigen helicase is very sensitive to actinomycin D, indicating significant differences between these helicases (Table 1). Our findings show other differences among prokaryotic, eukaryotic, and T antigen helicases, because the prokaryotic helicases are not blocked by distamycin bound to DNA (7), whereas both HeLa cell and T antigen helicases are blocked by distamycin. From the previously published comparisons of prokaryotic helicase sensitivities to drugs (5–7), from our earlier comparisons of T antigen and eukaryotic, HeLa, and FM-3A DNA helicases (4), and from our present comparisons of T antigen and HeLa helicase sensitivities to the epipodophyllotoxins teniposide and etoposide (Figs. 1 and 2), it is clear that significant differences exist among helicases in their sensitivities to different DNA-binding substances.

Because of their enzymatic mechanisms in DNA replication and transcription, helicases may be a particularly sensitive target for DNA-binding antibiotics, compared with topoisomerases. Topoisomerases attach to DNA at a site and catalyze duplex strand topological changes. Helicases attach to DNA and process along the duplex DNA strands according to their processivity characteristics. In theory, therefore, if an antibiotic molecule is bound to duplex DNA at a site, the topoisomerase may attach to a different site, not contact the antibiotic, and perform its topological function without interference. In contrast, helicase moves along the entire duplex DNA strand, traversing every site, and should encounter any antibiotic molecule bound to the duplex DNA. These differences in enzyme mechanisms may make helicases more susceptible to DNA-bound antibiotics. We find another difference between helicases and topoisomerase II in their responses to high concentrations of DNA-binding antibiotics. Unlike topoisomerase II, which may not be inhibited at high concentrations of intercalating drugs (14, 15), we find no decrease of helicase blockade at high drug concentrations. The helicases are directly and stoichiometrically sensitive to DNA binders.

DNA-intercalating antibiotics stabilize duplex DNA and increase the energy required to separate paired DNA strands (i.e., DNA melting) (17). The DNA-intercalating antibiotics daunorubicin (10), actinomycin D (11), elsamicin (12), and echinomycin (13) bind preferentially at G-C base pairs of duplex DNA, as do most natural intercalating antibiotics. G-C base pairs in duplex DNA have much greater electronic complementarity than do the A-T base pairs and are the most stable of the hydrogen-bonded base pairs, as seen in DNA melting. A reasonable assumption is that the maximal DNA helicase catalytic power is used to separate G-C base pairs. The combination of the high G-C base pair stability and the increased duplex DNA stability produced by an intercalated antibiotic at a G-C site increases the overall duplex DNA stability at the intercalator-G-C locus. This supernormal duplex DNA stability at the intercalator-G-C locus may exceed the catalytic power exerted by helicases to affect duplex DNA strand separation and may thus slow or stop helicase action. The factor of G-C base pair stability may have helped to determine the evolutionary selection of intercalating antibiotics for G-C-binding specificity.

The SV40 large T antigen helicase binds to our DNA substrate on the 3′ side of the duplex DNA, with subsequent 3′ to 5′ translocation along the DNA to produce strand dissociation (3). Possible modes of helicase inhibition by DNA-binding antibiotics are interference with the nonspecific helicase binding to the DNA single-strand and/or interference with the helicase translocation into the duplex DNA strands. Because the antibiotics we are testing show preferential binding to duplex DNA, we reason that the antihelicase action results from interference with helicase translocation into the duplex DNA region. We have also examined the possibility of direct action of the antibiotics on the helicase proteins, by comparing helicase reaction rates after preincubation of antibiotic with the enzymes, and we see no effect on the inhibition characteristics.

From our data on helicase blockade by DNA-binding anticancer antibiotics, we have documented an important action of these useful and important agents. The helicase process for the dissociation of duplex DNA strands is a necessary step for the cellular processes of DNA replication, transcription, and repair. Because each of these helicase-centered processes is crucial for the survival and propagation of the cells, it is possible that the inhibition of helicase activity by structurally disparate but functionally similar DNA-binding anticancer agents may explain their effectiveness against cancer cells.

Acknowledgment

We thank Merrill J. Egorin for generous support of our efforts and critical evaluation of our science.

References

1. Waring, M. J. DNA modification and cancer. *Annu. Rev. Biochem.* **50**:159–192 (1981).
2. Epstein, R. J. Drug-induced DNA damage and tumor chemosensitivity *J. Clin. Oncol.* **8**:2062–2084 (1990).
3. Matson, J. H., and K. A. Kaiser-Rogers. DNA helicases. *Annu. Rev. Biochem.* **59**:289–329 (1990).
4. Bachur, N. R., F. Yu, R. Johnson, R. Hickey, Y. Wu, and L. Malkas. Helicase inhibition by anthracycline anticancer agents. *Mol. Pharmacol.* **41**:993–998 (1992).
5. Maine, I. D., D. Sun, L. H. Hurley, and T. Kodadek. The antitumor agent CC-1065 inhibits helicase catalyzed unwinding of duplex DNA. *Biochemistry* **31**:3968–3975 (1992).
6. Sun, D., and L. Hurley. Structure activity relationships of (+)-CC-1065 analogues in the inhibition of helicase-catalyzed unwinding of duplex DNA. *J. Med. Chem.* **35**:1773–1782 (1992).
7. George, J. W., S. Ghate, S. W. Matson, and J. M. Besterman. Inhibition of DNA helicase II unwinding and ATPase activities by DNA-interacting ligands. *J. Biol. Chem.* **267**:10683–10689 (1992).
8. Simanis, V., and D. P. Lane. An immunoaffinity purification procedure for SV40 large T antigen. *Virology* **144**:88–100 (1985).
9. Malkas, L. R., R. Hickey, C. Li, N. Pederson, and E. F. Baril. A 21S enzyme complex from HeLa cells that functions in simian virus 40 DNA replication *in vitro*. *Biochemistry* **29**:6263–6274 (1990).
10. Chaires, J. B., J. E. Herrera, and M. J. Waring. Preferential binding of daunomycin to 5′ $^{A}_{T}$CG and 5′ $^{A}_{T}$GC sequences revealed by footprinting titration experiments. *Biochemistry* **29**:6145–6153 (1990).
11. Müller, W., and D. M. Crothers. Studies of the binding of actinomycin and related compounds to DNA. *J. Mol. Biol.* **35**:251–290 (1968).
12. Uesugi, M., T. Sekida, S. Matsuki, and Y. Sugiura. Selective DNA cleavage by elsamicin A and switch function of its amino sugar group. *Biochemistry* **30**:6711–6715 (1991).
13. Low, C. M. L., H. R. Drew, and M. J. Waring. Sequence-specific binding of echinomycin to DNA. *Nucleic Acids Res.* **12**:4865–4879 (1984).
14. Capranico, G., C. Soranzo, and F. Zunino. Single strand DNA breaks induced by chromophore-modified anthracyclines in P388 leukemia cells. *Cancer Res.* **46**:5499–5503 (1986).
15. Capranico, G., F. Zunino, K. Kohn, and Y. Pommier. Sequence-selective topoisomerase II inhibition by anthracycline derivative in SV40 DNA: relationship with DNA binding affinity and cytotoxicity. *Biochemistry* **29**:562–569 (1990).
16. Kopka, M. L., C. Yoon, D. Goodsell, P. Pjura, and R. E. Dickerson. The molecular origin of DNA-drug specificity in netropsin and distamycin. *Proc. Natl. Acad. Sci. USA* **82**:1376–1380 (1985).
17. Banville, D. L., M. A. Keniry, and R. H. Shafer. NMR investigation of mithramycin A binding to d(ATGCAT)$_2$. *Biochemistry* **29**:9294–9304 (1990).
18. Chow, K., T. L. Macdonald, and W. E. Ross. DNA binding by epipodophyllotoxins and *N*-acyl anthracyclines: implications for mechanism of topoisomerase II inhibition. *Mol. Pharmacol.* **34**:467–473 (1988).
19. Gabbay, E. J., D. Grier, R. E. Fingerle, R. Reimer, R. Levy, S. W. Pearce,

and W. D. Wilson. Interaction specificity of the anthracyclines with deoxyribonucleic acid. *Biochemistry* **15**:2062–2070 (1976).
20. Sobell, H. H., and S. C. Jain. Stereochemistry of actinomycin binding to DNA. *J. Mol. Biol.* **68**:21–34 (1972).
21. Graves, D. E., and T. R. Krugh. Adriamycin and daunorubicin bind in a cooperative manner to deoxyribonucleic acid. *Biochemistry* **22**:3941–3947 (1983).
22. Van Dyke, M. W., and P. B. Dervan. Chromomycin, mithramycin and olivomycin binding sites on heterogeneous deoxyribonucleic acid. *Biochemistry* **22**:2373–2377 (1983).
23. Youngquist, R. S., and P. B. Dervan. Sequence-specific recognition of B-DNA by oligo(*N*-methylpyrollecarboxamides). *Proc. Natl. Acad. Sci. USA* **82**:2565–2569 (1985).
24. diMarco, A., and F. Arcamone. DNA complexing antibiotics: daunomycin, Adriamycin and their derivatives. *Arzneim. Forsch.* **25**:368–375 (1975).
25. Meriwether, W. D., and N. R. Bachur. Inhibition of DNA and RNA metabolism by daunorubicin and Adriamycin in L1210 mouse leukemia. *Cancer Res.* **32**:1137–1142 (1972).
26. Ward, D. C., E. Reich, and I. H. Goldberg. Base specificity in the interaction of polynucleotides with antibiotic drugs. *Science (Washington D. C.)* **149**:1259–1263 (1965).
27. Sengupta, S. K., J. E. Anderson, Y. Kogan, D. H. Trites, W. R. Belt, and M. S. Madhavarao. N-2 and C-7 substituted actinomycin D analogues: synthesis, DNA binding affinity, and biochemical and biological properties: structure-activity relationship. *J. Med. Chem.* **24**:1052–1059 (1981).

Send reprint requests to: Nicholas R. Bachur, University of Maryland Cancer Center, 655 W. Baltimore Street, Room 9–021, Baltimore, MD 21201.