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Defining functional drug-interaction domains on topoisomerase II by exploiting mechanistic differences between drug classes

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Abstract. Topoisomerase II is the primary cellular target for a variety of antineoplastic drugs that are active against human cancers. These drugs exert their cytotoxic effects by stabilizing covalent topoisomerase II-cleaved DNA complexes that are fleeting intermediates in the catalytic cycle of the enzyme. Despite this common feature of drug action, a number of mechanistic differences between drug classes have been described. These mechanistic differences (including effects on DNA cleavage/religation, DNA strand passage, and adenosine triphosphate hydrolysis) were used as the basis for a series of competition experiments to determine whether different compounds share a common site of action on topoisomerase II or interact at distinct sites. Results of the present study strongly suggest that at least four structurally disparate antineoplastic drugs, etoposide, amsacrine, genistein, and the quinolone CP-115,953, share an overlapping interaction domain on the enzyme.

Key words: Drug-interaction domains – Topoisomerase II – DNA strand-break – DNA strand-passage

Introduction

Topoisomerase II is an essential enzyme that modulates the topological state of DNA in the cell by passing an intact DNA helix through a transient double-stranded break that it generates in a separate helix [27, 57]. In vivo, the enzyme plays important roles in virtually every aspect of DNA metabolism [27, 53, 57–59]. Moreover, topoisomerase II is required for proper chromosomal structure, condensation, and segregation [4, 27, 57].

Beyond its critical physiological functions, topoisomerase II is the target for a wide variety of antineoplastic drugs [4, 20, 31, 50]. Many of these compounds are highly active against human cancers, and drugs such as etoposide, doxorubicin, mitoxantrone, and amsacrine are used routinely for the treatment of these diseases [2, 12, 14, 30, 34, 52]. Although there is considerable structural diversity among topoisomerase II-targeted agents, these compounds share a common basis for their antineoplastic actions. They all stabilize covalent enzyme-cleaved DNA complexes that are requisite but fleeting intermediates in the catalytic cycle of topoisomerase II [4, 20, 29, 31, 58]. As a consequence of drug action, the physiological concentration of these DNA cleavage complexes rises dramatically following treatment of cells with topoisomerase II-targeted agents [23, 32, 33, 44, 45, 63, 64, 67]. Although DNA breaks formed in cleavage complexes are transient in nature, they are converted to permanent lesions when replication forks attempt to traverse these protein-bound roadblocks in the DNA [10, 49, 50, 66]. Consequently, cells that are treated with topoisomerase II-targeted drugs contain high levels of protein-associated breaks in their genomes. The presence of these breaks greatly stimulates the formation of chromosomal abnormalities [1, 11, 15, 22, 36] and ultimately triggers cell death by the process of apoptosis [16, 17]. Thus, topoisomerase II-targeted drugs act in an insidious fashion; they exert their cytotoxic effects not by inhibiting this essential enzyme but rather by converting it to a potent physiological toxin [4, 20, 31, 50].

Before the clinical applications of topoisomerase II as a target for cancer chemotherapy can be fully realized, it is

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Abbreviations: SDS, sodium dodecyl sulfate; APP(NH)P, adenyl-5′-yl-β,γ-imidodiphosphate

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critical to detail the mechanism of drug action. Clearly, in this regard our knowledge is still rudimentary. For example, for a number of years it was dogma that drugs stimulated enzyme-mediated DNA breakage by inhibiting the religation process [20, 50]. Furthermore, it was widely believed that these agents were specific for the cleavage/religation cycle of topoisomerase II. However, with the advent of assays that could monitor individual steps of the topoisomerase II catalytic cycle, it became obvious that this simplistic vision of drug action was no longer tenable. It is now clear that different classes of topoisomerase II-targeted drugs enhance DNA breakage by different means [4, 26, 37–40, 54, 55] and have a variety of effects on other steps of the catalytic cycle of the enzyme [6, 41].

One of the most important issues of drug mechanism that remains unresolved concerns the interaction domain(s) for drugs on topoisomerase II. To date, the only information concerning amino acid residues that are important for enzyme-drug interactions comes from the characterization of drug-resistant mutant type II topoisomerases (reviewed in [4]). However, it has yet to be demonstrated that any resistance-conferring mutation actually is located within the interaction domain on the enzyme for any given drug. In addition, generalizations regarding drug-interaction domains have been confounded by the observation that many mutant enzymes display different and often contradictory profiles of drug resistance. To illustrate this point, whereas the CEM/VM-1 enzyme displays resistance to all classes of DNA cleavage-enhancing drugs examined [8, 9], the HL-60/AMSA and KBM-3/AMSA enzymes show high resistance only to intercalative agents [68, 69]. Moreover, the Vpm^R-5 enzyme displays broad drug resistance but is highly sensitive to quinolones [39, 56]. Thus, in the absence of corroborative evidence, it is not obvious which (if any) DNA cleavage-enhancing drugs share a common site of action on topoisomerase II.

The present study describes some of the mechanistic differences between classes of topoisomerase II-targeted drugs. Furthermore, it presents a novel strategy that exploits these mechanistic difference to define drug-interaction domains on the enzyme. The results strongly suggest that a number of structurally diverse DNA cleavage-enhancing drugs share a common site of action on topoisomerase II.

Materials and methods

Materials. Topoisomerase II was purified from the nuclei of *Drosophila melanogaster* Kc tissue-culture cells by the procedure of Shelton et al. [51]. Negatively supercoiled pBR322 plasmid DNA was prepared by Triton X-100 lysis followed by banding in cesium chloride-ethidium bromide gradients [47]. Etoposide was obtained from Bristol Laboratories (Evansville, Ind.) or Sigma (St. Louis, Mo.), amsacrine was a generous gift from Dr. J. L. Nitiss (Children's Hospital of Los Angeles), genistein was obtained from ICN (Costa Mesa, Calif.), and the quinolones CP-115,953 and CP-80,080 were generous gifts from Drs. P. R. McGuirk and T. D. Gootz (Pfizer Central Research, Groton, Conn.).

Topoisomerase II-mediated DNA cleavage and religation. Assays were carried out as described by Robinson and Osheroff [38]. Samples contained 100 n*M* topoisomerase II and 5 n*M* negatively supercoiled pBR322 plasmid DNA in 20 µl assay buffer [10 m*M* TRIS-HCl (pH 7.9), 50 m*M* NaCl, 50 m*M* KCl, 5 m*M* $MgCl_2$, 0.1 m*M* ethylenediaminetetraacetic acid (EDTA), and 2.5% glycerol]. Pre-strand-passage reactions were carried out in the absence of a high energy cofactor, whereas post-strand-passage reactions included 1 m*M* APP(NH)P (Sigma, St. Louis, Mo.) [26, 38, 42]. DNA cleavage/religation equilibria were established for 6 min at 30° C. When DNA cleavage was monitored, products were trapped by the addition of SDS (1% final concentration) followed by EDTA (15 m*M* final concentration). When DNA religation was monitored, religation was induced by shifting samples from 30° C to 55° C for various times of up to 30 s prior to the addition of SDS. In all cases, topoisomerase II was digested with proteinase K (United States Biochemicals, Cleveland, Ohio; 60 µg/ml final concentration) for 45 min at 45° C. Reaction products were resolved by agarose-gel electrophoresis and levels of double-stranded DNA cleavage were quantitated by scanning densitometry as previously described [41].

DNA strand-passage event. The DNA strand-passage event mediated by topoisomerase II was monitored by the non-turnover DNA catenation assay of Corbett et al. [5]. In this assay, each molecule of topoisomerase II is capable of catalyzing only a single round of DNA strand passage. Briefly, reaction mixtures contained 100 n*M* topoisomerase II and 5 n*M* negatively supercoiled pBR322 DNA in 20 µl assay buffer that contained 6 µg histone H1/ml (Boehringer Mannheim, Indianapolis, Ind.) and 1 m*M* APP(NH)P. Samples were incubated at room temperature for 20 s, and reactions were stopped by the addition of EDTA (25 m*M* final concentration) followed by SDS (1 m*M* final concentration). Assay mixtures were digested with proteinase K as described above. Reaction products were resolved by agarose-gel electrophoresis and quantitated by scanning densitometry as previously described [6]. DNA strand passage was followed by monitoring the accumulation of high-molecular-mass catenenes or the loss of supercoiled plasmid substrate.

Topoisomerase II-catalyzed adenosine triphosphate hydrolysis. Adenosine triphosphatase (ATPase) assays were carried out as described by Osheroff et al. [28]. Reactions contained 10 n*M* topoisomerase II and 250 n*M* negatively supercoiled pBR322 plasmid DNA in a total of 20 µl assay buffer that contained 1 m*M* [γ-^{32}P]-ATP (Amersham, Arlington Heights, Ill.; 2 µCi/reaction). Mixtures were incubated at 30° C. At various intervals of up to 20 min, 2.5-µl samples were spotted onto thin-layer cellulose plates impregnated with polyethyleneimine (Polygram CEL 300 PEI, Brinkmann, Westbury, N.Y.) and chromatographed with freshly made 400 m*M* NH_4HCO_3. Radioactive areas corresponding to orthophosphate released by ATP hydrolysis were localized by autoradiography and quantitated by scintillation counting.

Results

Catalytic cycle of topoisomerase II

Fundamental to all physiological activities of topoisomerase II is the double-stranded DNA passage reaction [27, 57]. Before the effects of antineoplastic agents on enzyme activity can be analyzed appropriately and mechanistic differences between drug classes can be characterized, it is necessary to understand the mechanism by which topoisomerase II carries out its catalytic function.

The double-stranded DNA passage reaction of topoisomerase II can be separated into a number of discrete steps. These are shown schematically in Fig. 1, which depicts one round of the catalytic cycle of the enzyme (reviewed in [4, 29]). In step 1, topoisomerase II (*1*) binds

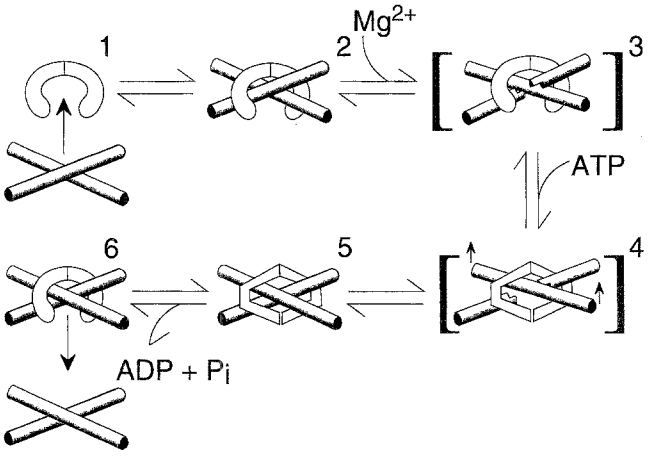

Fig. 1. Catalytic scheme for topoisomerase II-mediated DNA strand passage. One round of the catalytic cycle of the enzyme is shown. The DNA substrate is represented by *cylinders* and the homomeric enzyme, by *handlebar-shaped structures*. Transient topoisomerase II-DNA cleavage complexes [pre- (*3*) and post-strand passage (*4*)] are shown in *brackets*. The change in enzyme structure in complexes 4 and 5 represents the structural transition that occurs upon ATP binding [18]. In this scheme, topoisomerase II binds to DNA (*1*⇋2), establishes a pre-strand-passage DNA cleavage/religation equilibrium (*2*⇋3), mediates double-stranded DNA passage (*3*⇋4), establishes a post-strand-passage DNA cleavage/religation equilibrium (*4*⇋5), hydrolyzes ATP (*5*⇋6), and recycles to regenerate *1*

DNA at points of helix-helix juxtaposition [43, 65] to form a noncovalent complex (*2*). In step 2, topoisomerase II establishes a DNA cleavage/religation equilibrium [21, 25, 48]. During cleavage, the enzyme forms a covalent attachment with the newly generated 5′-DNA termini via an O^4-phosphotyrosyl linkage [46, 61]. The transient pre-strand-passage topoisomerase II-DNA cleavage complex (*3*) is shown in brackets. In step 3, ATP binding induces a conformational change in the enzyme [18]. This structural transition promotes the DNA strand-passage event in which the intact helix is translocated through the DNA break [24, 28, 42]. The resulting post-strand-passage cleavage complex (*4*) is also shown in brackets. In step 4, the enzyme establishes a post-strand-passage DNA cleavage/religation equilibrium [24, 38]. In step 5, ATP within the noncovalent post-strand-passage topoisomerase II-DNA complex (*5*) is hydrolyzed [19, 28]. This in turn regenerates the pre-strand-passage conformation of the enzyme (*6*) and triggers enzyme turnover (i.e., recycling) [24, 42].

Mechanistic differeces between classes of topoisomerase II-targeted drugs

Although it was widely speculated that all antineoplastic drugs enhanced topoisomerase II-mediated DNA cleavage by inhibiting religation, it was not until the late 1980s, when DNA religation-specific assays were developed, that this hypothesis became testable. Initial studies that analyzed the effects of etoposide [26, 38] and amsacrine [37, 38] on the kinetics of DNA religation appeared to confirm the speculation that all topoisomerase II-targeted drugs acted by inhibiting the religation activity of the enzyme.

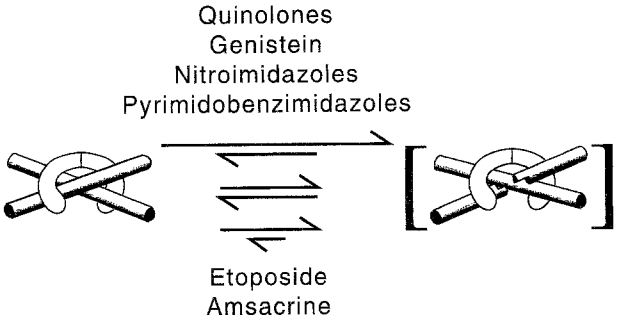

Fig. 2. Mechanism by which topoisomerase II-targeted drugs enhance DNA breakage. The DNA substrate is represented by *cylinders* and the enzyme, by *handlebar-shaped structures*. The transient topoisomerase II-DNA cleavage complex is shown in *brackets*. Etoposide [26, 38] and amsacrine [37, 38, 55] act primarily by inhibiting topoisomerase II-mediated DNA religation. Quinolones [39, 40], genistein [4], nitroimidazoles [54, 55], and pyrimido[1,6-*a*]benzimidazoles [7] act primarily by stimulating the rate of DNA cleavage

Table 1. Effects of drugs on selected steps of the topoisomerase II catalytic cycle[a]

Reaction step	Drug				
	Etoposide	Amsacrine	Genistein	CP-115,953	Novobiocin
Pre-strand-passage DNA breakage[b]	+++	++	+++	+++	0
Pre-strand-passage religation	↓↓↓	↓↓↓	0	↓/0	NA[c]
DNA strand passage	↓/0	↓↓	↓↓↓	↓↓	↓↓↓
Post-strand-passage DNA breakage[b]	++	+	++	++	↓/0
Post-strand-passage religation	↓↓↓	↓↓↓	0	0/+	NA[c]
ATP hydrolysis	0	↓↓	↓↓↓	↓	↓↓↓

[a] The effects of antineoplastic drugs ranged from strongly inhibitory (↓↓↓), to no effect (0), to strongly stimulatory (+++). *Drosophila* topoisomerase II was used for all experiments. Data from [4, 6, 37–39, 41]

[b] This denotes the level of DNA cleavage at equilibrium of the cleavage/religation step

[c] Not applicable

However, in 1991 the universality of this hypothesis was severely undermined when it was discovered that the novel quinolone CP-115,953 [6,8-difluoro-7-(4′-hydroxyphenyl)-1-cyclopropyl-4-quinolone-3-carboxylic-acid] and related compounds strongly enhanced topoisomerase II-mediated DNA cleavage without significantly affecting DNA religation [39, 40]. For example, under conditions in which both CP-115,953 and etoposide enhanced DNA cleavage ~8-fold, CP-115,953 decreased the apparent first-order rate of pre-strand-passage topoisomerase II-mediated DNA religation by less than 15% (as compared with ~90% for etoposide) and actually increased the rate of post-strand-passage religation by ~40% (as compared with an inhibition of ~90% by etoposide) [39]. These findings are despite the fact that the quinolone is a more potent effector of

the eukaryotic type II enzyme than is etoposide [13, 39]. Thus, it appears that a second mechanism for drug action, stimulation of the forward DNA cleavage event, must exist. Since the initial report on quinolones, as least three additional drug classes have been found to utilize this latter mechanism [4, 7, 54, 55]. Data obtained for a number of compounds are summarized in Fig. 2 and Table 1.

Given that different classes of drugs utilize alternative mechanisms for enhancing DNA breakage, recent studies [6, 41] have addressed mechanistic differences for other steps in the catalytic cycle of topoisomerase II (Table 1). The compounds that were used in these studies are representative of a broad spectrum of drug classes including demethylepipodophyllotoxins (etoposide), anilinoacridines (amsacrine), isoflavones (genistein), and quinolones (CP-115,953). In addition to their structural diversity, these agents utilize different DNA-binding modes, with amsacrine being intercalative in nature [60] and the others being nonintercalative [3, 39, 62].

The results indicate significant differences between drug classes (Table 1). In the most dramatic illustration, genistein strongly inhibits both the DNA strand-passage and ATP hydrolysis steps, whereas etoposide has little effect on either. Furthermore, although amsacrine and CP-115,953 utilize different mechanisms to stimulate topoisomerase II-mediated DNA breakage, they have similar effects on DNA strand passage and ATP hydrolysis. Two major conclusions can be drawn from these studies. First, the actions of topoisomerase II-targeted drugs are not necessarily confined to the DNA cleavage and religation events catalyzed by the enzyme. Second, each drug class acts with its own individual profile. Among the drug classes examined to date (admittedly limited in number), no two agents have displayed the same mechanistic fingerprint. Furthermore, no correlation has been found between either the mode of drug-DNA binding or the effects of compounds on DNA religation and the ability to inhibit any other reaction step.

Table 2. Defining drug-interaction domains on topoisomerase II by competition experiments[a]

Drug	Effect of etoposide on drug-induced inhibition of DNA strand passage[b]		Effect of CP-80,080 on drug-induced enhancement of DNA cleavage[c]	
	–Etoposide (%)	+Etoposide (%)	–CP-80,080 (%)	+CP-80,080 (%)
No drug	100	86	100	130
Etoposide[d]	–	–	530	170
Amsacrine[e]	49	91	380	190
Genistein[f]	0	82	350	140
CP-115,953[g]	43	83	540	170

[a] Enzyme activity was set to 100% in the absence of drug
[b] 500 μ*M* etoposide was used
[c] 400 μ*M* CP-80,080 was used
[d] The concentration of etoposide used in DNA cleavage assays was 50 μ*M*
[e] The concentration of amsacrine used in DNA cleavage and strand-passage assays was 500 and 75 μ*M*, respectively
[f] The concentration of genistein used in DNA cleavage and strand-passage assays was 250 and 100 μ*M*, respectively
[g] The concentration of CP-115,953 used in DNA cleavage and strand-passage assays was 500 and 50 μ*M*, respectively

Defining functional interaction domains for antineoplastic drugs on topoisomerase II

Genetics studies have defined a number of amino acid mutations that confer drug resistance to topoisomerase II (reviewed in [4]). Thus far, these mutations have appeared to cluster in two regions. One region (residues 450–499 in the human type II enzyme) is located in the gyrB homology domain of topoisomerase II and spans one of the two consensus ATP recognition motifs in the enzyme. The other region (residues 760–907) is located in the gyrA homology domain and spans the active-site tyrosine that cleaves DNA.

Despite the available genetic information, it remains unclear whether DNA cleavage-enhancing drugs share a common site of action on topoisomerase II. Since virtually every mutant reported has displayed a unique drug-resistance profile [4], different conclusions concerning interaction domains can be drawn from each study. Moreover, since several mutant type II topoisomerases have obvious changes in their enzymatic properties [9] and/or stability [56], it is probable that resistance (in at least some cases) results from global changes in enzyme structure rather than from specific alterations in drug-binding sites.

To characterize more fully interactions between antineoplastic drugs and topoisomerase II, a biochemical approach that defines relationships between drug-interaction domains on the enzyme has been developed [6, 41]. Although this technique does not identify amino acid residues in topoisomerase II that are involved in drug binding, it can readily determine whether the interaction domains for two compounds overlap one another or are distinct. Thus, the biochemical and genetic approaches complement one another.

The biochemical approach takes advantage of mechanistic differences between drug classes such as those described in Table 1. These differences are exploited to design a series of competition experiments that categorize drug-interaction domains on topoisomerase II. Since this method is based on drug function, interaction domains elucidated by this approach are termed functional interaction domains.

Thus far, three studies defining functional drug-interaction domains on topoisomerase II have been carried out. The first addressed relationship between the interaction domain for DNA cleavage-enhancing drugs and novobiocin (data not shown) [41]. This coumarin-based drug has no effect on the DNA cleavage/religation equilibrium of the enzyme [41] and was originally described as a compound that was an inhibitor of topoisomerase II-catalyzed ATP hydrolysis (Table 1) [28, 35]. On the basis of the following observations, it was concluded that the interaction domain for novobiocin was distinct from those of DNA cleavage-enhancing antineoplastic drugs:

1. Novobiocin was incapable of blocking the ability of etoposide, amsacrine, genistein, or CP-115,953 to stimulate enzyme-mediated DNA cleavage. For exam-

ple, a decrease of less than 2% in etoposide (100 μ*M*)-promoted DNA cleavage was observed upon addition of novobiocin (250 μ*M*).
2. Etoposide (500 μ*M*) was incapable of reversing the inhibition of either DNA strand passage or ATP hydrolysis induced by the presence of novobiocin (250 μ*M*).

The second study addressed relationships between the interaction domains for etoposide on topoisomerase II and those of other DNA cleavage-enhancing drugs [6]. In this study, the ability of etoposide (which inhibits the DNA strand-passage step of the topoisomerase II catalytic cycle by less than 15% at concentrations as high as 500 μ*M*; Table 1) to reverse the inhibition of DNA strand passage by amsacrine, genistein, and CP-115,953 was characterized. The results are shown in Table 2. In all cases, etoposide reversed the effects of the other drugs on the DNA strand-passage event. In the most dramatic case, etoposide (500 μ*M*) restored the level of strand passage observed in the presence of genistein (250 μ*M*; no activity) to greater than 80% activity. These results strongly suggest that the functional interaction domain of etoposide overlaps those of amsacrine, genistein, and CP-115,953. However, it is not clear from this study whether the interaction domains for these other DNA cleavage-enhancing drugs also overlap one another.

Therefore, to determine whether antineoplastic drugs other than etoposide share a common site of action on topoisomerase II, the third study defined the functional interaction domain for quinolones on the enzyme. Competition experiments took advantage of the previous finding that the quinolone CP-80,080 [6-fluoro-7-(4′-methoxyphenyl)-1-cyclopropyl-4-quinolone-3-carboxylic acid] interacts with eukaryotic topoisomerase II but is a poor enhancer of DNA cleavage. (At a concentration of 400 μ*M*, CP-80,080 stimulates DNA cleavage ~1.3-fold.) They also took advantage of the observation that this quinolone does not in itself inhibit the DNA scission activity of the enzyme. The latter point is critical because it demonstrates that CP-80,020 does not block any enzyme function prior to cleavage.

The functional interaction domain for quinolones on topoisomerase II was analyzed by determining the ability of CP-80,080 to prevent the enhancement of DNA breakage by etoposide, amsacrine, genistein, and CP-115,953. As seen in Table 2, the quinolone was capable of competing with all drugs tested. In all cases, levels of drug-stimulated DNA cleavage were reduced by more than 50% when CP-80,080 was included in assays. Similar results were obtained when CP-80,080 was replaced by ciprofloxacin (data not shown). Like CP-80,080, this potent quinolone-based antibiotic (targeted to the prokaryotic type II topoisomerase, DNA gyrase [35]) is a weak enhancer of DNA cleavage [13]. These data provide evidence that the interaction domain for quinolones on topoisomerase II overlaps those of etoposide, amsacrine, and genistein. Taken together with the results of the etoposide competition experiments, these findings strongly suggest that all (or at least several classes of) DNA cleavage-enhancing antineoplastic drugs share a common site of action on the eukaryotic type II enzyme.

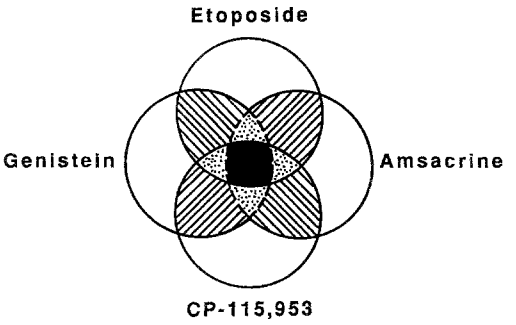

Fig. 3. Schematic representation of the overlapping interaction domains on topoisomerase II for etoposide, amsacrine, genistein, and the quinolone CP-115,953. The degree of overlap between domains is indicated by *shading*. *White*, No overlap; *hatched*, overlap between two domains; *stippled*, overlap between three domains; *black*, overlap between all four domains. The intersection between the interaction domains of amsacrine and genistein is indicated as a *dotted line* because it has not yet been demonstrated that these two drugs share a common binding site on topoisomerase II

Discussion

All topoisomerase II-targeted antineoplastic drugs act by increasing the cellular concentration of enzyme-DNA cleavage complexes [4, 20, 31, 50]. Despite this common denominator, different drug classes display distinct mechanistic profiles [4]. Whereas some agents enhance topoisomerase II-mediated DNA breakage primarily by inhibiting the religation reaction of the enzyme [26, 37, 38, 55], others appear to act by stimulating the forward rate of cleavage [4, 7, 38, 39, 54, 55]. In addition, several agents have differential effects on a number of steps of the catalytic cycle of topoisomerase II [6, 41].

What are the clinical ramifications of this mechanistic diversity among classes of topoisomerase II-targeted antineoplastic agents? The answer is currently unknown. However, several issues deserve future attention. First, the mechanism by which DNA breakage is enhanced impacts the longevity of the enzyme-DNA cleavage complex (i.e., drugs that inhibit religation promote cleavage complexes with much longer half-lives than do compounds that act by increasing the rate of cleavage). Therefore, given two agents that increase the cellular concentration of topoisomerase II-DNA cleavage complexes to a comparable extent, will the drug that produces the longer-lived DNA breaks prove to be more lethal? Second, a drug that impairs religation may be more likely to produce chromosome breaks, whereas a compound that enhances rates of DNA cleavage may be more likely to promote intermolecular DNA ligation events. Thus, does the mechanism of drug action impact the clastogenic versus recombinational properties of that agent? Third, compounds that block ATP hydrolysis severely impair enzyme turnover and the subsequent release of DNA by topoisomerase II [29]. As a result, ATPase inhibitors trap the enzyme on its DNA target [24, 42]. Therefore, would the ability to inhibit ATP hydrolysis increase the cytotoxic potential of DNA cleavage-enhancing drugs?

Finally, the present study exploited mechanistic differences between drug classes to define functional drug-in-

teraction domains on topoisomerase II. The results make it likely that the interaction domains for a number of antineoplastic drugs overlap one another [6]. This conclusion is depicted schematically for etoposide, amsacrine, genistein, and CP-115,953 in the circle diagram shown in Fig. 3. The degree of overlap between interaction domains is indicated by the different shadings. In light of Fig. 3, it is clear how different resistance-conferring mutations can produce such different drug-resistance profiles, even though antineoplastic agents appear to share a common site on the enzyme. At least 13 different profiles can be envisioned using the simplistic diagram shown in Fig. 3. These range from mutations that are specific for an individual drug class to those that display broad resistance. Therefore, the enzymological approach utilized in the present work provides a novel framework in which to interpret the results of genetics studies. Since the enzymological and genetic approaches provide complementary information, when used in conjunction they should create a powerful tool for defining drug-interaction domains on topoisomerase II.

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