A Two-drug Model for Etoposide Action against Human Topoisomerase IIα*

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The widely used anticancer drug etoposide kills cells by increasing levels of topoisomerase II-mediated DNA breaks. While it is known that the drug acts by inhibiting the ability of topoisomerase II to ligate cleaved DNA molecules, the precise mechanism by which it accomplishes this action is not well understood. Because there are two scissile bonds per enzyme-mediated double-stranded DNA break, it has been assumed that there are two sites for etoposide in every cleavage complex. However, it is not known whether the action of etoposide at only one scissile bond is sufficient to stabilize a double-stranded DNA break or whether both drug sites need to be occupied. An oligonucleotide system was utilized to address this important issue. Results of DNA cleavage assays support a two-drug model for the action of etoposide against human topoisomerase IIα. This model postulates that drug interactions at both scissile bonds are required in order to increase enzyme-mediated double-stranded DNA breaks. Etoposide actions at either of the two scissile bonds appear to be independent of one another, with each individual drug molecule stabilizing a strand-specific nick rather than a double-stranded DNA break. This finding suggests (at least in the presence of drug) that there is little or no communication between the two promoter active sites of topoisomerase II. The two-drug model has implications for cancer chemotherapy, the cellular processing of etoposide-stabilized enzyme-DNA cleavage complexes, and the catalytic mechanism of eukaryotic topoisomerase II.

Etoposide is a highly successful anticancer drug that has been in clinical use for nearly 20 years (1). It is employed as frontline therapy for a variety of human malignancies, including leukemias, lymphomas, and several solid tumors (1–3). Until the emergence of the taxanes, etoposide was the most widely prescribed anticancer drug in the world.

The primary cellular target for etoposide is topoisomerase II (1, 2, 4–8). This essential enzyme alters DNA topology by passing an intact double helix through a transient double-stranded break that it generates in a separate nucleic acid segment (4, 5, 7–11). The enzyme is required to resolve knots and tangles in the genetic material that are produced by normal cellular processes such as DNA recombination and replication (4, 5, 9, 10, 12, 13). In the absence of topoisomerase II, cells are unable to segregate daughter chromosomes and die of mitotic failure (5, 9, 12).

Eukaryotic topoisomerase II is homodimeric in nature (14–18). Each protomer contains an active site tyrosine (Tyr-805 in human topoisomerase IIα (19)) that is responsible for cleaving one strand of the double helix (14, 20, 21). In order to maintain the integrity of the genetic material during the double-stranded DNA passage reaction of topoisomerase II, each active site residue forms a covalent phosphotyrosyl bond with one newly generated 5′ terminus (14, 20, 21). This covalent topoisomerase II-cleaved DNA complex is known as the cleavage complex. Despite the physiological importance of topoisomerase II, the cleavage complex is potentially toxic. When DNA-tracking enzymes such as polymerases or helicases attempt to traverse the topoisomerase II roadblock on the genetic material, transient cleavage complexes can be converted to permanent double-stranded DNA breaks (4–6, 8, 22). Normally, cleavage complexes are present at very low levels and are tolerated by the cell. However, conditions that increase the cellular concentration of topoisomerase II-associated nucleic acid breaks lead to DNA recombination and mutagenesis (4–6, 23–26). When these breaks overwhelm the cell, they trigger programmed death pathways (6, 26).

In contrast to most drugs that target specific enzymes, etoposide and other topoisomerase II-targeted anticancer agents act in an insidious manner. Rather than blocking the activity of this essential enzyme, etoposide kills cells by increasing the concentration of topoisomerase II-DNA cleavage complexes (2, 4–8). This action converts topoisomerase II to a potent cellular toxin that fragments the genome. Consequently, etoposide is referred to as a topoisomerase II poison to distinguish it from drugs that inhibit the overall catalytic activity of the enzyme (2, 4–7, 27, 28).

It has been known for more than a decade that etoposide stabilizes topoisomerase II-associated double-stranded DNA breaks by inhibiting the ability of the enzyme to ligate cleaved nucleic acid molecules (4, 5, 8, 29–31). However, the molecular mechanism by which it does so is unknown.

Topoisomerase II manufactures double-stranded breaks in DNA by generating two staggered nicks (one on each strand) in the sugar-phosphate backbone (14, 20, 21). The fact that there are two scissile bonds per double-stranded break implies that there are two available sites for drug action in every cleavage complex. The potential presence of two etoposide molecules raises a fundamental question regarding drug mechanism. Does topoisomerase II require the actions of etoposide at both scissile bonds in order to stimulate the formation of double-stranded DNA breaks, or does the presence of drug at either scissile bond suffice? If enzyme catalysis at both strands is

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The two-drug model has implications for future drug design and the cellular processing of etoposide-stabilized cleavage complexes.

**Experimental Procedures**

Enzymes and Materials—Wild-type human topoisomerase IIα and a mutant human enzyme containing an active site Phe in place of Tyr-805 (Y805F) were expressed in Saccharomyces cerevisiae and purified as described previously (32–34). Etoposide (Sigma) was prepared as a 20 mM stock solution in 100% Me2SO and stored at 4 °C. Preparation of Oligonucleotides—A 47-base oligonucleotide corresponding to residues 80–126 of pBR322 and its complement were prepared on an Applied Biosystems DNA synthesizer. The sequences of the top and bottom strands were 5′- CGGATCATCTCCGAGGCT-3′ and 5′-ACGGTGCCGAGGATGACCAGT GACCAGT GACCGGTAGATTGCATCCTCGGCACCGT-3′, respectively. Points of topoisomerase II-mediated DNA cleavage are denoted by arrows. Oligonucleotides spanning the 5′ terminus to the point of topoisomerase II scission on each strand were synthesized. Single-stranded oligonucleotides were labeled on their 5′ termini with γ-32P-phosphate and purified as described (35). Oligonucleotides extending from the point of scission to the 5′ terminus of each strand were synthesized and 5′-activated with p-nitrophenol according to Bromberg et al. (34). Equimolar amounts of complementary oligonucleotides were annealed by incubating at 70 °C for 10 min and cooling to 25 °C.

DNA Cleavage—DNA cleavage assays were carried out as described previously (33, 36). Reaction mixtures contained 200 nM wild-type human topoisomerase IIα and 10 nM double-stranded oligonucleotide or 50 nM enzyme and 10 nM negatively supercoiled pBR322 DNA in 20 μl of 10 mM Tris-HCl, pH 7.9, 135 mM KCl, 5 mM MgCl2, 0.1 mM EDTA, and 2.5% glycerol in the absence or presence of etoposide. Reactions were incubated at 37 °C for 15 min. For mixtures containing oligonucleotide substrates, cleavage intermediates were trapped and digested as above, with the addition of 2 μl of 10% SDS followed by 1 μl of 375 mM EDTA, pH 8.0. Samples were digested with proteinase K and ethanol-precipitated. To monitor strand-specific double-stranded DNA breaks, cleavage products were resolved by electrophoresis in 1% agarose gel in 40 mM Tris acetate, pH 8.3, 2 mM EDTA. To monitor single-stranded DNA breaks, cleavage products were resolved by electrophoresis in 7 M urea, 14% polyacrylamide gels in 100 mM Tris borate, pH 8.3, 2 mM EDTA. To monitor double-stranded DNA breaks, products were resolved in 12% nondenaturing polyacrylamide gels. In all cases, DNA cleavage products were visualized and quantified on a Bio-Rad Molecular Imager.

For reactions containing pBR322 DNA, cleavage intermediates were trapped and digested as above (except that 5% SDS was used) and subjected to electrophoresis in 1% agarose gel in 40 mM Tris acetate, pH 8.3, 2 mM EDTA containing 0.5 mM ethidium bromide. DNA bands were visualized by ultraviolet light and quantified using an Alpha Innotech digital imaging system. Relative levels of single-versus double-stranded breaks were determined directly by quantifying the fluorescence of linear and nicked plasmid molecules, respectively (ethidium bromide displays identical binding and fluorescence properties for both linear forms of DNA and nicked DNA (37)). These values were converted to absolute cleavage levels by normalizing data to the total percent DNA cleavage, which was calculated from the loss of supercoiled plasmid DNA substrate.

DNA Ligation—DNA ligation reactions were carried out according to Bromberg et al. (34). Briefly, assays contained 200 nM wild-type or Y805F human topoisomerase IIα and 10 nM activated nicked oligonucleotide in a total of 20 μl of 10 mM Tris-HCl, pH 7.9, 135 mM KCl, 7.5 mM CaCl2, 0.1 mM EDTA, and 2.5% glycerol in the absence or presence of etoposide. Reaction mixtures were incubated at 37 °C for 45 h, and ligation was stopped by the addition of 2 μl of 10% SDS followed by 1 μl of 375 mM EDTA, pH 8.0. Samples were treated, resolved in denaturing polyacrylamide gels, and analyzed as described above for oligonucleotide cleavage reactions.

**Results**

Although etoposide kills cells by stabilizing topoisomerase II DNA cleavage complexes (2, 4–8), the precise mechanism by which it accomplishes this action remains enigmatic. Because this drug is in wide use for the treatment of human cancers (1–3), it is critical to understand the molecular basis for its effects on the enzyme.

Because there are two scissile bonds per topoisomerase II-mediated double-stranded DNA break (14, 20, 21), it has been assumed that there are two sites for etoposide in every cleavage complex (38). However, it is not known whether the presence of etoposide at only one scissile bond is sufficient to stabilize a double-stranded DNA break or whether both drug sites need to be occupied. This is a fundamental issue that has important implications for topoisomerase II-based cancer chemotherapy, as well as drug design and the cellular processing of drug-stabilized cleavage complexes.

Two potential models for etoposide action can be envisioned. In the first, or one-drug model, the action of etoposide at either of the two scissile bonds is sufficient to produce enzyme-mediated double-stranded DNA breaks. This model postulates strong molecular communication between the active site of each topoisomerase II protomer, such that drug-induced inhibition of ligation of one DNA strand also impairs ligation of the other strand.

In the second, or two-drug model, the action of etoposide at both of the two scissile bonds is required to generate double-stranded DNA breaks. This model postulates that there is little or no molecular communication between the two protomer active sites of topoisomerase II. Each drug acts independently, inhibits DNA ligation only at a single scissile bond, and therefore induces only a single-stranded DNA break.

Although these hypotheses have not been tested for any eukaryotic type II topoisomerase, results of a study that examined drug effects on bacteriophage T4 topoisomerase II (38) were consistent with the one-drug model. Under all conditions examined in this work, primarily double-stranded DNA breaks were induced by etoposide. However, because treatment of eukaryotic type II topoisomerases with anticancer drugs often produces cleavage complexes containing DNA nicks (29, 39–41), the one-drug model may not extend to the eukaryotic enzymes.

**Treatment of Human Topoisomerase IIα with Etoposide Stimulates the Formation of Single-stranded DNA Breaks—**

The one-drug model of etoposide action predicts that drug treatment will produce enzyme-associated DNA breaks that are primarily double-stranded. Conversely, the two-drug model predicts that etoposide and未必会generally primarily single-stranded breaks at subsaturating drug concentrations. In addition, the ratio of single-stranded to double-stranded DNA breaks will drop with rising drug concentration, because the probability that both protomer active sites of topoisomerase II are occupied by drug will increase at higher levels of etoposide. The latter two predictions appear to be true for human topoisomerase IIα (Fig. 1). At the lowest drug concentration examined (50 μM), the ratio of single-stranded to double-stranded DNA breaks was 2.6:1.1 Furthermore, this ratio dropped to approximate parity at 500 μM etoposide. Although the experiment shown in Fig. 1 was carried out in the absence of a high

Because the agarose gel system employed to resolve pBR322 topoisomers cannot distinguish between molecules that contain single or multiple nicks, the ratio of single-stranded to double-stranded DNA breaks probably is underestimated.
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energy cofactor, similar results were observed when ATP or a nonhydrolyzable ATP analog was included in DNA cleavage assays (not shown). Taken together, these findings are inconsistent with the one-drug model and provide support for the two-drug model discussed above.

Etoposide Acts Independently at the Two Scissile Bonds—A postulate of the two-drug model is that the action of etoposide at one scissile bond does not influence the DNA cleavage/ligation equilibrium at the other. This implies that there is little or no communication between the protomer active sites of topoisomerase II, at least in the presence of the drug.

This prediction cannot be tested using a plasmid-based DNA cleavage or ligation assay, because it is impossible to control the distribution of sites utilized by the enzyme in large nucleic acid substrates. Consequently, an oligonucleotide system was established that allowed the actions of etoposide at each of the two scissile bonds to be manipulated individually (see Fig. 2). The substrate contained a single, well characterized cleavage site for topoisomerase II that was derived from pBR322 plasmid DNA (14, 42, 43). The cleavage site did not fit the “consensus sequence” for etoposide (C at the \(-1\) position relative to the point of scission) (44, 45), but rather contained a \(-1\) G on both the top and bottom strands. However, equilibrium levels of scission on each individual strand increased nearly 30-fold at 500 \(\mu M\) drug (Fig. 3, A and C).

As a first step toward manipulating the efficacy of etoposide at the individual scissile bonds, the nucleotide preference of the drug for the base at the \(-1\) position was characterized on both strands of the oligonucleotide substrate. The \(-1\) G of the top strand was changed to an A, T, or C (a corresponding change was made in the complementary strand to maintain base-pairing), and the level of single-stranded breaks in this strand was monitored. As predicted from previous sequence analysis (44, 45), the greatest drug-induced stimulation of DNA cleavage of the top strand (\(-70\)-fold at 500 \(\mu M\) drug) was observed when the base at the \(-1\) position was a C (Fig. 3A). A similar increase in the level of single-stranded breaks in the bottom strand was seen when the \(-1\) G was changed to a C (Fig. 3C). The nucleotide preference for inhibition of DNA ligation by

Fig. 2. Oligonucleotides used to examine etoposide action. The central sequence of the duplex substrate (residues 80–126 of pBR322) used to monitor DNA cleavage (\(A\) and \(C\)) by wild-type human topoisomerase II or DNA ligation (\(B\) and \(D\)) by either the Y805F or the wild-type enzyme is shown. Points of topoisomerase II-mediated DNA scission are denoted by the arrows. Asterisks indicate the radiolabeled DNA strands on which strand-specific DNA cleavage and ligation were monitored. Substrates for DNA ligation contained a nick at the point of scission for topoisomerase II, and the terminal 5’-phosphate at the nick was activated for DNA ligation by the covalent attachment of 5′-nitropheno (34). Oligonucleotides \(A–D\) represent the substrates utilized in the corresponding (panels) (\(A–D\)) of Figs. 3–5. When appropriate, the G at the \(-1\) position (in bold) relative to the point of scission on the top or bottom strand was changed to an A, T, or C.

Fig. 3. Sequence specificity of etoposide. DNA substrates used in \(A–D\) are as shown in Fig. 2. DNA cleavage mediated by wild-type human topoisomerase IIa (\(A\)) and ligation by the Y805F mutant enzyme (\(B\)) were monitored on the top strand. DNA cleavage mediated by wild-type human topoisomerase IIa (\(C\)) and ligation by the Y805F mutant enzyme (\(D\)) were monitored on the bottom strand. The \(-1\) base (relative to the point of scission for topoisomerase II) on the strand being cleaved/ligated was changed as indicated. For all substrates, the amount of DNA cleavage observed in the absence of etoposide was set to 1 (\(A\) and \(C\)), and the amount DNA ligation observed in the absence of drug was set to 100% (\(B\) and \(D\)). All substrates contained a weak drug site (\(-1\) G) on opposite strand. Error bars indicate the standard deviation of three independent experiments.
etoposide was assessed by monitoring the ability of human topoisomerase IIα to seal a nicked oligonucleotide whose 5'-phosphate terminus was activated by covalent attachment to the tyrosine mimic, p-nitrophenol (34). To ensure that results were not influenced by potential drug-induced cleavage on the strand opposite to that being monitored, a Y805F active site mutant of human topoisomerase IIα (that was incapable of mediating DNA scission) (34) was used for these initial experiments.

The effects of nucleotide sequence on etoposide-induced inhibition of DNA ligation were striking (Fig. 3, A and B). When the −1 G on either strand was converted to a C, the IC_{50} for etoposide-induced inhibition of DNA ligation on that strand decreased 20–25-fold (Table I). These results supply further evidence that etoposide raises the concentration of topoisomerase II-DNA cleavage complexes primarily by inhibiting DNA ligation.

A previous study that examined interactions between etoposide and yeast topoisomerase II determined that the affinity of the drug for the enzyme decreased ∼3-fold when the active site tyrosine was replaced by a phenylalanine (46). Therefore, the above DNA ligation experiments were repeated using wild-type human topoisomerase IIα in place of the Y805F mutant enzyme. Qualitatively, results were the same (Table I). The IC_{50} value for etoposide-induced inhibition of DNA ligation mediated by wild-type topoisomerase IIα on either strand decreased ∼30-fold when the −1 G on the strand being monitored was converted to a C. However, as predicted by the previous drug–enzyme binding data, DNA ligation mediated by the wild-type enzyme was always 2–5 times more sensitive to inhibition by etoposide than the Y805F mutant (Table I).

The DNA cleavage and ligation data presented above provide a framework in which the effect of etoposide on each scissile bond can be manipulated individually. Therefore, two sets of experiments were carried out to determine whether the action of etoposide at one scissile bond alters the DNA cleavage/ligation equilibrium at the other. The first monitored enzyme-mediated DNA cleavage and ligation at a scissile bond containing a weak etoposide site (∼1 G), and the second monitored these reactions at a scissile bond containing a strong drug site (∼1 C). If etoposide action at one scissile bond is communicated to the other, introduction of a strong drug site on the opposite strand should have a significant effect on DNA cleavage and ligation of the strand being monitored. Conversely, if etoposide acts independently at the two scissile bonds, introduction of a strong etoposide site on the opposite strand should have little or no effect on the DNA strand being monitored.

It should be noted that wild-type human topoisomerase IIα rather than the Y805F mutant enzyme was used to examine potential etoposide-induced interstrand communication during DNA ligation. Because the Y805F mutant enzyme cannot cleave DNA, it is not clear whether etoposide is capable of acting on the strand opposite the activated nick in ligation assays that employ this enzyme. Wild-type topoisomerase IIα, however, establishes a DNA cleavage/ligation equilibrium on the nonactivated strand at the same time that it catalyzes a unidirectional DNA ligation reaction on the activated strand. Under the conditions examined, etoposide stimulation of DNA cleavage on the nonactivated strand of a ligation substrate (see oligonucleotides B and D in Fig. 2) was similar to that observed for the corresponding strand of an intact DNA cleavage substrate (see oligonucleotides A and C in Fig. 2) (data not shown).

In the first set of experiments, DNA cleavage and ligation mediated by wild-type human topoisomerase IIα were monitored at a scissile bond containing a weak etoposide site (−1 G) when the opposite strand contained either a weak (−1 G) or a strong (−1 C) site for drug action. As seen in Fig. 4, A and B, enhancement of DNA cleavage and inhibition of DNA ligation at the weak etoposide site on the top strand were unaffected when the bottom strand was converted to a strong drug site. Although a marginal increase in cleavage of the bottom strand (Fig. 4C) was observed when the top strand was converted to a strong drug site, there was no effect on the inhibition of ligation (Fig. 4D).

In the second set of experiments, DNA cleavage and ligation were monitored at a scissile bond containing a strong etoposide site (−1 C) when the opposite strand contained either a weak or a strong drug site. In all cases, the introduction of a strong drug site on the opposite strand had (at best) a marginal effect on the ability of etoposide to increase cleavage at the strong site being

### Table I

| DNA strand | Enzyme | IC_{50} etoposide | DNA strand | Enzyme | IC_{50} etoposide |
|------------|--------|------------------|------------|--------|------------------|
| Top        | Y805F  | 80 µM            | Bottom     | Y805F  | 55 µM            |
| Wild-type  | 24     | 0.8              | Wild-type  | 32     | 1.1              |

a Base at the −1 position relative to the point of scission for topoisomerase IIα.

**Fig. 4. Effect of a strong etoposide site on the DNA cleavage/ligation equilibrium of a weak drug site on the opposite strand.** DNA substrates used in A–D are as shown in Fig. 2. A and B, DNA cleavage and ligation mediated by wild-type human topoisomerase IIα were monitored on the top strand. C and D, DNA cleavage and ligation by wild-type human topoisomerase IIα were monitored on the bottom strand. All substrates contained a weak drug site (−1 G) on the cleaved/ligated strand. The −1 base on the opposite strand was changed from a G to a C as indicated to create a strong etoposide site. For all substrates, the amount of DNA cleavage observed in the absence of etoposide was set to 1 (A and C), and the amount DNA ligation observed in the absence of drug was set to 100% (B and D). Error bars indicate the S.D. of three independent experiments.
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Fig. 5. Effect of a strong etoposide site on the DNA cleavage/ligation equilibrium of a strong drug site on the opposite strand. DNA substrates used in A–D are as shown in Fig. 2. A and B, DNA cleavage and ligation mediated by wild-type human topoisomerase IIa were monitored on the top strand. C and D, DNA cleavage and ligation by wild-type human topoisomerase IIa were monitored on the bottom strand. All substrates contained a strong drug site (−1 G) on the cleaved/ligated strand. The −1 base on the opposite strand was changed from a G to a C as indicated to create the strong etoposide site. The insets in B and D expand the data shown for enzyme-mediated DNA ligation carried out in the presence of low concentrations (0–10 μM) of etoposide. For all substrates, the amount of DNA cleavage observed in the absence of etoposide was set to 1 (A and C), and the amount DNA ligation observed in the absence of drug was set to 100% (B and D). Error bars represent the S.D. of three independent assays.

These results indicate that the action of etoposide at one scissile bond has little or no influence on the DNA cleavage/ligation equilibrium at the other scissile bond. They further suggest that the actions of etoposide at the two scissile bonds of a topoisomerase II-DNA cleavage complex are independent of one another and provide strong evidence for a two-drug model in which there is little or no communication between the two protomer active sites of topoisomerase II.

Enhanced Formation of Double-stranded DNA Breaks Requires Strong Etoposide Sites on Both DNA Strands.—If the proposed model for etoposide action on human topoisomerase IIα is correct, etoposide should increase levels of enzyme-mediated double-stranded DNA breaks only when both scissile bonds are occupied with drug. To test this critical aspect of the model, topoisomerase II-generated double-stranded DNA breaks were monitored by resolving reaction products in nondenaturing gels. The following conditions were examined: both strands of the DNA cleavage sequence contained a weak etoposide site (G at the −1 position); only one of the two strands (either the top or the bottom) contained a strong drug site (C at the −1 position); or both strands contained a strong drug site. All experiments utilized the oligonucleotide system described in the previous section. Results are shown when reactions were carried out in the presence of 50 or 500 μM etoposide.

Fig. 6. Enhanced formation of double-stranded DNA breaks requires strong etoposide sites on both DNA strands. DNA cleavage reactions contained either 50 (left) or 500 μM (right) etoposide (Etop). Double-stranded DNA breaks (dsDNA breaks) were monitored when both strands of the oligonucleotide substrate contained weak drugs sites (Both −1 G), only one of the two strands contained a strong drug site (Top −1 C or Bottom −1 C), or both strands contained strong drug sites (Both −1 C). In order to quantify double-stranded DNA breaks, cleavage products were resolved under nondenaturing conditions. Error bars represent the S.D. of three to four independent assays.

Fig. 7. Two-drug model for etoposide action against human topoisomerase IIα. In order to induce double-stranded DNA breaks, etoposide (red) interactions are required at both scissile bonds of a topoisomerase II-DNA cleavage complex (bottom). Etoposide actions at either of the two scissile bonds (arrows) are independent of one another, with each individual drug molecule stabilizing a strand-specific nick (left or right) rather than a double-stranded break. There appears to be little or no molecular communication between the two protomer active sites of topoisomerase II (green ovals) in the presence of etoposide. See text for further details.

Results support the proposed two-drug model (Fig. 6). When a strong etoposide site (−1 C) was introduced on either the top or bottom DNA strand at either drug concentration, levels of double-stranded breaks increased slightly (<60% on average). However, when strong sites were introduced on both strands, levels of double-stranded DNA breaks rose dramatically, −5.5-fold at 50 μM etoposide and −3.5-fold at 500 μM drug.
DISCUSSION

A Two-drug Model for Etoposide Action—On the basis of the data presented above, we propose a two-drug model for the actions of the anticancer drug etoposide against human topoisomerase IIa. This model, which is depicted in Fig. 7, postulates that separate drug molecules must act at each of the two scissile bonds of a cleavage complex in order to increase enzyme-mediated double-stranded DNA breaks. Etoposide actions at either of the two scissile bonds appear to be independent of one another, with each individual drug molecule stabilizing a strand-specific nick rather than a double-stranded DNA break. A final implication of this model is that there is little or no communication between the two protomer active sites of topoisomerase II, at least in the presence of etoposide.

The two-drug model for etoposide is supported by several lines of evidence. First, etoposide treatment generated a high ratio of single-stranded to double-stranded DNA breaks at low drug concentrations, and this ratio fell as drug concentration (and presumably etoposide occupancy in the cleavage complex) rose. Second, the action of etoposide on one strand of a cleavage complex had no appreciable effect on the DNA cleavage/ligation ratio of single-stranded to double-stranded DNA breaks at low concentrations at either of the two scissile bonds appear to be independent of one another, with each individual drug molecule acting at each of the two scissile bonds (47–49). In addition, several models similar to those proposed for the conversion of transient single-stranded topoisomerase I-associated DNA breaks to permanent double-stranded breaks in camptothecin-treated cells (6, 50–52) may be more appropriate.

Beyond the role of topoisomerase II in cancer chemotherapy, there is substantial evidence suggesting that maternal consumption of topoisomerase II-active agents triggers chromosomal translocations in the MLL oncogene (chromosomal band 11q23) that lead to specific de novo infant leukemias (25, 53–57). Although the chromosomal breakpoints in these malignancies are located near topoisomerase II-DNA cleavage sites, the genomic rearrangements in the MLL oncogene are complex and often contain large insertions or deletions. It has been proposed that these translocations are initiated by the formation of multiple topoisomerase II-DNA cleavage complexes that generate nicks (rather than double-stranded breaks) on opposite strands of the double helix (56). Although the precise mechanism by which leukemic chromosomal translocations are generated has yet to be delineated, the two-drug model is compatible with this proposal.

At the present time, the two-drug model is being proposed specifically for etoposide. While there is a presumption (albeit untested) that it will hold for other drugs that poison topoisomerase II by inhibiting enzyme-mediated DNA ligation, it is not clear whether the model can be applied to agents that act by stimulating the forward rate of DNA scission. It has been proposed that drugs in this latter category enhance topoisomerase II-mediated DNA breaks by disturbing the double helix proximal to the scissile bond (8, 43, 58, 59). Consequently, it is possible that the presence of a single drug molecule will be sufficient to induce this distortion and stimulate the formation of a topoisomerase II-associated double-stranded DNA break.

Finally, the fact that drug-induced changes in the DNA cleavage/ligation equilibrium at one scissile bond are not communicated to the other implies that the two protomer active sites of eukaryotic topoisomerase II work in a fashion that is less coordinated than previously assumed. Recently, it has been noted that type IA and eukaryotic type II topoisomerases share structural elements in their catalytic domains (60). This observation, coupled with biochemical studies (61, 62), has led to the proposal that these enzymes share a common ancestral DNA nicking-closing mechanism (60, 62). The potential independence between the two active sites of eukaryotic topoisomerase II is consistent with such an hypothesis and merits further investigation.

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