Quinolone Action against Human Topoisomerase IIα: Stimulation of Enzyme-Mediated Double-Stranded DNA Cleavage†

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ABSTRACT: Several important antineoplastic drugs kill cells by increasing levels of topoisomerase II-mediated DNA breaks. These compounds act by two distinct mechanisms. Agents such as etoposide inhibit the ability of topoisomerase II to ligate enzyme-linked DNA breaks. Conversely, compounds such as quinolones have little effect on ligation and are believed to stimulate the forward rate of topoisomerase II-mediated DNA cleavage. The fact that there are two scissile bonds per double-stranded DNA break implies that there are two sites for drug action in every enzyme−DNA cleavage complex. However, since agents in the latter group are believed to act by locally perturbing DNA structure, it is possible that quinolone interactions at a single scissile bond are sufficient to distort both strands of the double helix and generate an enzyme-mediated double-stranded DNA break. Therefore, an oligonucleotide system was established to further define the actions of topoisomerase II-targeted drugs that stimulate the forward rate of DNA cleavage. Results indicate that the presence of the quinolone CP-115,953 at one scissile bond increased the extent of enzyme-mediated scission at the opposite scissile bond and was sufficient to stimulate the formation of a double-stranded DNA break by human topoisomerase IIα. These findings stand in marked contrast to those for etoposide, which must be present at both scissile bonds to stabilize a double-stranded DNA break [Bromberg, K. D., et al. (2003) *J. Biol. Chem. 278*, 7406−7412]. Moreover, they underscore important mechanistic differences between drugs that enhance DNA cleavage and those that inhibit ligation.

Knots and tangles that accumulate within the genetic material are potentially lethal to the cell. These topological barriers are removed from the genome by an essential enzyme known as topoisomerase II (*1*−*7*).

Topoisomerase II acts by passing an intact segment of duplex DNA through a transient double-stranded break that it generates in a separate double helix (*1*−*6*, *8*, *9*). The enzyme manufactures the double-stranded break by generating two staggered nicks (one on each strand) in the sugar−phosphate backbone (*10*, *11*). This scission reaction yields cleaved DNA molecules containing four-base single-stranded cohesive ends protruding on their 5′-termini (*11*). To maintain chromosomal integrity during the double-stranded DNA passage reaction, topoisomerase II forms covalent attachments with the newly created 5′-DNA termini via its two active site tyrosyl residues (one per each protomer subunit) (*10*−*12*). This covalent enzyme-cleaved DNA intermediate is known as the *cleavage complex*.

Even though cells cannot survive without the double-stranded DNA passage activity of topoisomerase II, the formation of cleavage complexes is potentially toxic. If DNA tracking enzymes such as polymerases or helicases encounter a cleavage complex, they can convert these transient topoisomerase II intermediates into permanent double-stranded DNA breaks (*3*, *4*, *13*−*15*). The presence of these breaks initiates mutagenic events, including chromosomal insertions, deletions, and translocations (*15*−*19*), and can trigger cell death pathways (*15*, *16*).

Beyond its required physiological activities, topoisomerase II is the primary target for a number of antineoplastic drugs, several of which are used for the clinical treatment of human cancers (*3*, *4*, *13*, *15*, *20*−*22*). Although topoisomerase II-targeted agents encompass a number of structurally diverse classes, they all kill cells by elevating the level of enzyme-generated DNA breaks (*3*, *4*, *13*, *15*, *20*−*22*). Since these drugs convert the essential type II enzyme to a cellular toxin, they are termed "topoisomerase II poisons" to distinguish them from agents that inhibit the double-stranded DNA passage reaction of the enzyme (*3*, *4*, *13*, *15*, *20*−*24*).

Topoisomerase II poisons increase equilibrium levels of DNA cleavage complexes by two distinct, but not mutually exclusive, mechanisms. Drugs such as etoposide (and derivatives), amsacrine, and TAS-103 act primarily by inhibiting the ability of topoisomerase II to reseal (ligate) enzyme-linked DNA breaks (*3*, *4*, *13*, *25*−*28*). Conversely, drugs such as CP-115,953 (and other quinolones), ellipticine, and genistein have little effect on DNA ligation and appear to act primarily by stimulating the forward rate of topoisomerase

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II-mediated DNA scission (*3*, *4*, *13*, *28*−*30*). Furthermore, a variety of DNA lesions (including abasic sites and alkylated bases) also poison the enzyme without affecting rates of ligation (*31*−*33*).

It is believed that compounds that inhibit DNA ligation interfere with the ability of topoisomerase II to align the 3′- and/or 5′-DNA termini generated by enzyme-mediated cleavage (*13*). The fact that there are two scissile bonds per enzyme-generated double-stranded DNA break implies that there are two available sites for drug action (one on each strand) in every topoisomerase II−DNA cleavage complex. A previous study that examined drug effects on human topoisomerase IIα indicated that etoposide actions at both scissile bonds are required to stabilize double-stranded breaks in the genetic material (*34*). The presence of etoposide at either scissile bond inhibits DNA ligation only on that strand, and hence stabilizes a single-stranded break in the double helix (*34*).

Since drugs such as quinolones have little effect on the DNA ligation reaction of eukaryotic type II enzymes, it is not clear whether the "two-drug model" described above for etoposide can be applied to agents that increase the level of DNA breaks by stimulating the forward rate of topoisomerase II-mediated scission. On the basis of studies with position-specific DNA lesions (*32*, *33*, *35*), it is believed that compounds in this latter group enhance scission by locally distorting the structure of DNA, thus creating a preferable cleavage substrate for the enzyme (*13*, *32*, *36*). Consequently, it is possible that quinolone interactions at a single scissile bond are sufficient to distort both strands of the double helix and stimulate the formation of an enzyme-mediated double-stranded DNA break. In support of this possibility, the presence of strand-specific DNA lesions dramatically increases levels of topoisomerase II-generated double-stranded breaks in the genetic material (*32*, *35*).

Therefore, an oligonucleotide system was established to further define the actions of topoisomerase II poisons that stimulate the forward rate of DNA cleavage. Results with human topoisomerase IIα indicate that the presence of the quinolone CP-115,953 at a single scissile bond is sufficient to stimulate enzyme-mediated cleavage on both strands of the double helix. This finding stands in marked contrast to the requirements for the generation of double-stranded DNA breaks by etoposide (*34*). Moreover, it further underscores the mechanistic differences between drugs that enhance DNA cleavage and those that inhibit ligation.

EXPERIMENTAL PROCEDURES

Enzymes and Materials. Wild-type human topoisomerase IIα and a mutant human enzyme containing an active site Phe in place of Tyr805 (Y805F) were expressed in *Saccharomyces cerevisiae* using the YEpWOB6 vector and purified as described previously (*37*−*39*). CP-115,953 (Pfizer Global Research) was prepared as a 20 mM stock solution in 100% DMSO 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′-CCGTGTATGAAATCTAACAATG↓CGCTCATCG-TCATCCTCGGCACCGT-3′ and 5′-ACGGTGCCGAGGA-TGACGATG↓AGCGCATTGTTAGATTTCATACACGG-3′, respectively. Points of topoisomerase II-mediated DNA cleavage are denoted with arrows. Oligonucleotides spanning the 5′-terminus to the point of topoisomerase II scission on the top and bottom strands also were synthesized, and single-stranded oligonucleotides were labeled on their 5′-termini with [^{32}P]phosphate and purified as described previously (*40*). Oligonucleotides extending from the point of scission to the 3′-terminus of each strand were synthesized and 5′-activated with *p*-nitrophenol according to the method of Bromberg et al. (*39*). 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 (*38*, *41*). Reaction mixtures contained 200 nM wild-type human topoisomerase IIα and 10 nM double-stranded oligonucleotide or 20 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 $MgCl_2$, 0.1 mM EDTA, and 2.5% glycerol in the absence or presence of CP-115,953. Reaction mixtures were incubated at 37 °C for 15 min. For mixtures containing oligonucleotide substrates, reactions were stopped by adding 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 single-stranded DNA breaks, cleavage products were resolved by electrophoresis under denaturing conditions in 7 M urea, 14% polyacrylamide gels in 100 mM Tris-borate (pH 8.3), and 2 mM EDTA. To monitor double-stranded DNA breaks, products were resolved in 12% nondenaturing polyacrylamide gels. In both cases, DNA cleavage products were visualized and quantified on a Bio-Rad FX Molecular Imager.

For reaction mixtures containing pBR322 DNA, cleavage intermediates were trapped and digested as described above (except that 5% SDS was used) and subjected to electrophoresis in a 1% agarose gel in 40 mM Tris-acetate (pH 8.3) and 2 mM EDTA containing 0.5 μg/mL ethidium bromide. DNA bands were visualized with ultraviolet light and quantified using an Alpha Innotech digital imaging system. Levels of single-stranded and double-stranded DNA breaks and absolute cleavage levels were quantified as described previously (*34*).

DNA Ligation. DNA ligation reactions were carried out according to the method of Bromberg et al. (*39*). Briefly, assays contained 200 nM 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 $CaCl_2$, 0.1 mM EDTA, and 2.5% glycerol in the absence or presence of CP-115,953. Reaction mixtures were incubated at 37 °C for 48 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 processed, resolved in denaturing polyacrylamide gels, and analyzed as described above for oligonucleotide cleavage reactions.

RESULTS AND DISCUSSION

The Quinolone CP-115,953 Has Little Effect on the DNA Ligation Reaction of Human Topoisomerase IIα. Previous studies indicate that quinolones have little, if any, effect on the ability of *Drosophila* topoisomerase II to ligate DNA

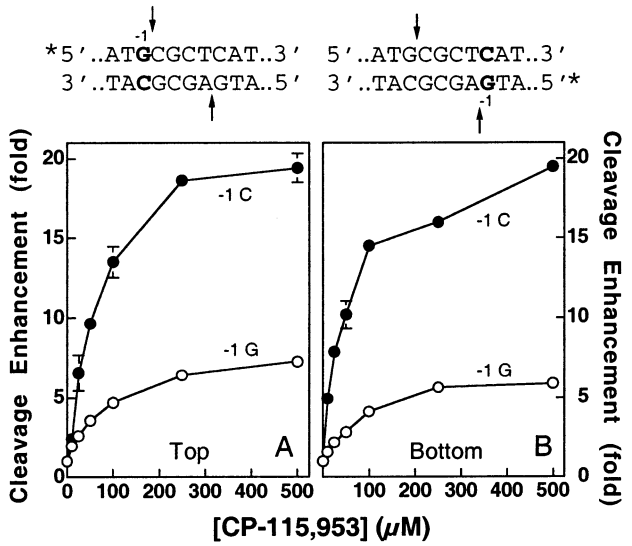

FIGURE 1: Enhancement of DNA cleavage by CP-115,953. The central sequence of the oligonucleotide substrate used to monitor DNA cleavage by wild-type human topoisomerase IIα on either the top strand (A) or the bottom strand (B) is shown. The asterisks denote the radiolabeled strands on which DNA cleavage was monitored, and the points of topoisomerase II scission are denoted with arrows. The −1 base relative to the point of scission on the strand being cleaved was changed from a G to a C as indicated. The amount of DNA scission observed in the absence of CP-115,953 was set to 1. Error bars indicate the standard deviation of two to three independent experiments.

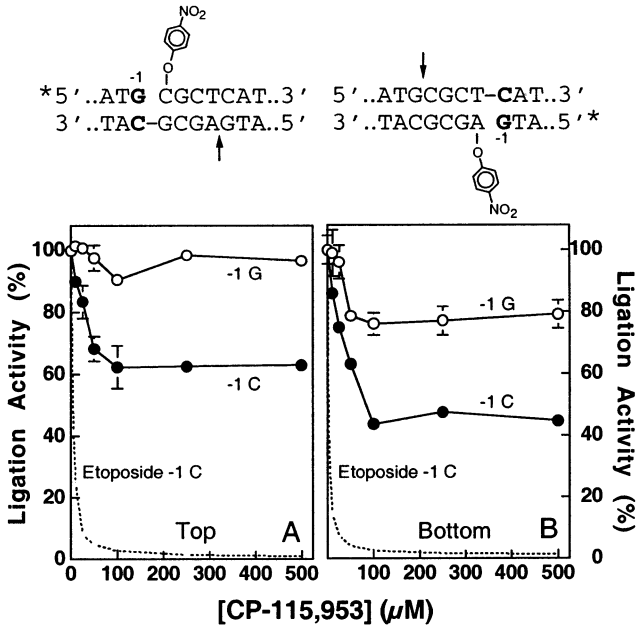

FIGURE 2: CP-115,953 has little effect on the DNA ligation reaction of human topoisomerase IIα. The central sequence of the oligonucleotide substrate is per Figure 1 except that the strand being ligated contained a nick at the topoisomerase II point of scission and was activated for DNA ligation by the attachment of *p*-nitrophenol to the 5′-terminal phosphate (*39*). DNA ligation mediated by Y805F human topoisomerase IIα was monitored on either the top (A) or the bottom (B) strand. The −1 base relative to the point of scission on the strand being ligated was changed from a G to a C as indicated. The amount of DNA ligation observed in the absence of CP-115,953 was set to 100%. The dashed lines represent the effect of etoposide on DNA ligation (from ref *34*). Error bars indicate the standard deviation of three to four independent experiments.

(*29*, *42*). Under conditions in which etoposide inhibited ligation rates more than 10-fold, CP-115,953 slowed ligation rates by <30% (*29*, *42*). In contrast to results with the *Drosophila* enzyme, quinolones strongly inhibit the DNA ligation reaction of the prokaryotic type II enzyme, topoisomerase IV (*43*, *44*). Because the mechanistic basis for quinolone action is not strictly conserved across evolutionary boundaries, the effect of CP-115,953 on the DNA ligation activity of human topoisomerase IIα was determined.

An oligonucleotide system was employed for these experiments. The substrate contained a single, well-characterized cleavage site for topoisomerase II that was derived from pBR322 plasmid DNA (*11*, *32*, *45*). Statistical analysis of DNA sequences cleaved by topoisomerase II in the presence of CP-115,953 suggests that the quinolone prefers sites that contain a C at the −1 position (relative to the point of scission) (*46*, *47*). Although the parental oligonucleotide substrate contained a G at the −1 position, CP-115,953 increased equilibrium levels of cleavage on each strand ~5-fold (Figure 1A,B). However, consistent with the predicted consensus sequence for the drug, when the G at the −1 position was converted to the preferred C (a corresponding change was made in the complementary strand to maintain base pairing), the quinolone increased scission on each strand ~20-fold (Figure 1A,B).

The effect of CP-115,953 on the DNA ligation activity of human topoisomerase IIα was assessed by monitoring the ability of the enzyme to seal a nicked oligonucleotide whose 5′-phosphate terminus was activated by covalent attachment to the tyrosine mimic, *p*-nitrophenol (*39*). To ensure that results were not influenced by potential drug-induced cleavage on the strand opposite from that being ligated, the Y805F active site mutant of human topoisomerase IIα that was incapable of mediating DNA scission (*39*) was used for these experiments.

As seen in panels A and B of Figure 2, CP-115,953 had little effect on topoisomerase II-mediated DNA ligation. When the oligonucleotide substrate contained a G at the −1 position, less than 25% inhibition [as compared to the 5-fold increase in equilibrium levels of DNA cleavage (Figure 1)] was observed on either strand at saturating drug concentrations (~100 μM). No further inhibition was evident, even at quinolone levels that were 5 times higher. The effect of CP-115,953 on DNA ligation was only marginally greater when the G at the −1 position was converted to the preferred C. Approximately 50% inhibition was observed on either strand at 100 μM drug, with no further increase at higher quinolone concentrations. Once again, this minor inhibition cannot account for the 20-fold increase in the levels of topoisomerase II-generated DNA breaks observed in Figure 1.

These results are in direct contrast to those found for etoposide (*34*). This latter drug dramatically impaired the ability of human topoisomerase IIα to ligate the activated oligonucleotide substrate (Figure 2A,B). When the sequence contained the preferred C at the −1 position on the top or bottom strand, the IC_{50} value for etoposide was 4 or 2 μM, respectively. Furthermore, nearly 98% inhibition was observed at 100 μM drug. On the basis of these findings, we conclude that the quinolone CP-115,953 has little or no effect on the ability of human topoisomerase IIα to ligate DNA

and probably increases the level of enzyme-associated DNA breaks primarily by stimulating the forward rate of DNA cleavage.

CP-115,953 Action on One Strand Alters the DNA Cleavage/Ligation Equilibrium on the Opposite Strand. A previous study indicated that etoposide, a topoisomerase II-targeted anticancer drug that blocks DNA ligation, must act at both scissile bonds to stabilize a double-stranded DNA break (*34*). Inhibition of ligation at one scissile bond by the drug had no effect on the DNA cleavage/ligation equilibrium at the other bond (*34*).

Unlike etoposide, CP-115,953 does not significantly impair the ability of topoisomerase II to seal DNA breaks. Therefore, it is not clear that similar results would be expected for the quinolone. On the basis of work with strand-specific DNA lesions (*32*, *33*, *35*), it has been proposed that drugs that enhance the forward rate of enzyme-mediated DNA scission act by distorting the double helix within the cleavage site (*13*, *32*, *36*). This structural perturbation is recognized by both protomer active sites of topoisomerase II, and therefore increases the rate of DNA scission on both strands by creating a preferred nucleic acid conformation for cleavage. Since the distortion is lost when the double-stranded recognition site is converted to single-stranded overhangs by the cleavage event (see Figure 5), normal rates of ligation are observed in the presence of drug.

Quinolones perturb the structure of double-stranded DNA proximal to the site of cleavage (*48*−*50*). Moreover, this perturbation is considerably stronger in the presence of prokaryotic and eukaryotic type II enzymes (*48*−*50*). Therefore, as shown for DNA lesions (*32*, *35*), it is predicted that the action of CP-115,953 on only one of the two strands of a cleavage site would be sufficient to generate a double-stranded break in the genetic material. As a first step toward testing this hypothesis, the oligonucleotide cleavage substrate described in the previous section was used to determine whether the interaction of the quinolone on one DNA strand altered the cleavage/ligation equilibrium on the opposite strand.

As seen in panels A and B of Figure 3, conversion of the G at the −1 position on either the top or bottom strand to the preferred C had a significant effect on levels of cleavage observed on the opposite strand. For either substrate, a 2−3-fold increase in the extent of DNA scission was apparent.

The experiments described above monitored DNA cleavage on a strand containing a "weak" (i.e., −1 G) site for CP-115,953. Studies that examined the effects of DNA damage on topoisomerase II indicate that the introduction of a second lesion within a cleavage site further increases levels of scission over those seen with a single lesion (*35*, *51*). Presumably, the presence of the second lesion creates an even greater distortion in the double helix. Therefore, an additional set of experiments was carried out to determine whether the interaction of CP-115,953 on the top strand (for example) affects levels of cleavage on the bottom strand, when the bottom strand contains a "strong" quinolone site (i.e., −1 C). As shown in panels C and D of Figure 3, conversion of the G at the −1 position on either the top or bottom strand to the preferred C increased the level of DNA cleavage 1.5−2-fold on the opposite strand, even though the opposite strand already contained a strong site (−1 C) for CP-115,953.

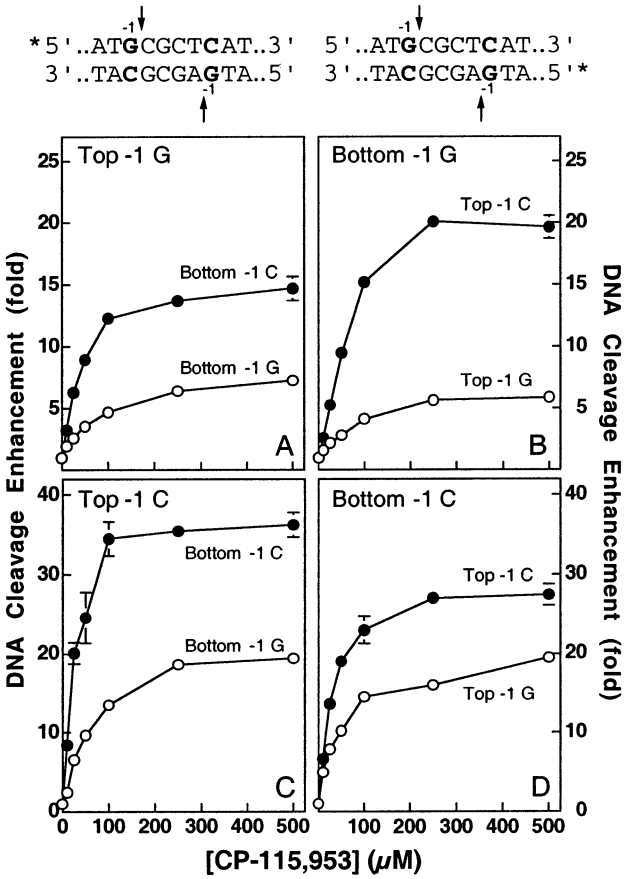

FIGURE 3: Action of CP-115,953 on one strand alters the DNA cleavage/ligation equilibrium on the opposite strand. DNA cleavage mediated by wild-type human topoisomerase IIα was monitored on the top (left) or the bottom (right) strand. The central sequence of the cleavage substrate is per Figure 1. In panels A and B, the stand being monitored contained a weak CP-115,953 site (−1 G), and the base at the −1 position on the opposite strand was converted from a weak (−1 G) to a strong site (−1 C) as indicated. In panels C and D, the strand being monitored contained a strong CP-115,953 site (−1 C), and the base at the −1 position on the opposite strand was converted from a weak (−1 G) to a strong site (−1 C) as indicated. The amount of DNA scission observed in the absence was CP-115,953 was set to 1. Error bars represent the standard deviation of two to four independent assays.

Taken together, these results strongly suggest that the presence of CP-115,953 on one strand of the double helix stimulates the ability of human topoisomerase IIα to cleave the scissile bond on the opposite strand.

The Action of CP-115,953 on One DNA Strand Is Sufficient To Stimulate the Formation of a Topoisomerase II-Mediated Double-Stranded DNA Break. The above findings imply that the action of CP-115,953 at either scissile bond should increase levels of double-stranded DNA breaks generated by human topoisomerase IIα. This hypothesis was examined by analyzing the DNA products of cleavage reactions on nondenaturing gels, as opposed to the denaturing gels used for Figure 3.

The effects of CP-115,953 on double-stranded DNA cleavage were monitored over a range of 50−500 μM drug when the oligonucleotide substrate contained weak (−1 G) quinolone sites on both DNA strands, a strong site (−1 C) on either the top or bottom strand, or strong sites on both strands (Figure 4A). At all quinolone concentrations that were tested, levels of double-stranded DNA breaks were ∼2-fold

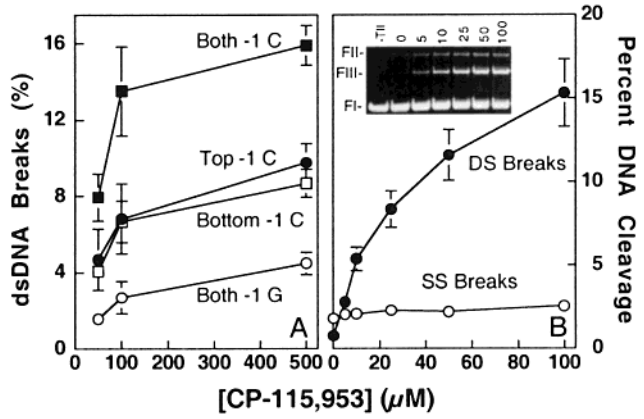

FIGURE 4: Generation of double-stranded DNA breaks by CP-115,953. (A) Double-stranded DNA breaks (dsDNA breaks) generated by wild-type human topoisomerase IIα were monitored when both strands of the oligonucleotide substrate contained weak drugs sites (Both −1 G), only one of the two strands contained a strong drug site (Top −1 C or Bottom −1 C), or both strands contained strong drug sites (Both −1 C). To quantify double-stranded DNA breaks, cleavage products were resolved under nondenaturing conditions. (B) The percentages of single-stranded (SS Breaks) and double-stranded (DS Breaks) DNA breaks generated by human topoisomerase IIα were monitored in pBR322 DNA. The inset shows an ethidium bromide-stained agarose gel of a typical DNA cleavage assay containing 0−100 μM quinolone. Single-stranded and double-stranded DNA cleavage convert negatively supercoiled plasmid DNA (form I, FI) to nicked circular (form II, FII) and linear molecules (form III, FIII), respectively. Error bars represent the standard deviation of two to four independent experiments.

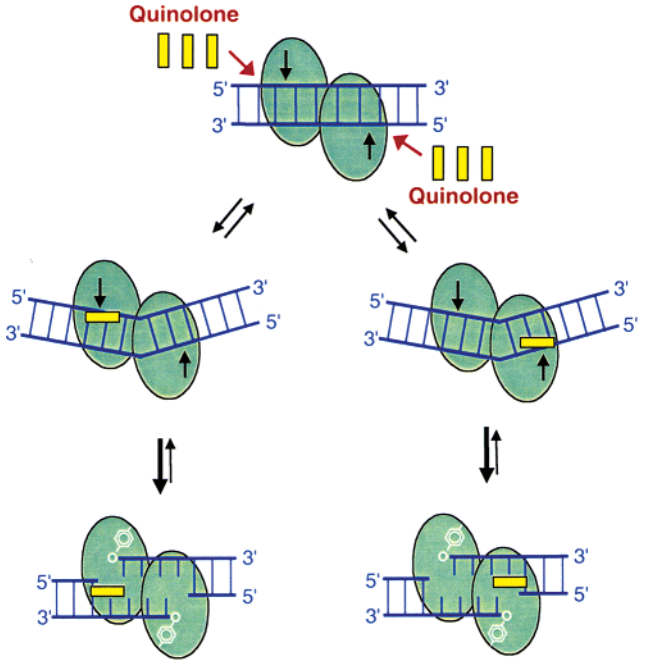

FIGURE 5: Model of quinolone action against human topoisomerase IIα. The presence of quinolone on either DNA strand perturbs the structure of the double helix, generating a preferable substrate for DNA scission. This distortion is recognized by both protomer subunits of topoisomerase II, and therefore increases the rate of DNA scission on both strands. Consequently, the interaction of quinolone at only one scissile bond stimulates the formation of an enzyme-mediated double-stranded DNA break.

higher when either the top or bottom strand was converted to a strong site for CP-115,953. Moreover, as predicted from the data in panels C and D of Figure 3, a further enhancement (∼2-fold) of double-stranded DNA cleavage was observed when the substrate contained strong quinolone sites on both strands.

As a final test of the above hypothesis, the concentration dependence of CP-115,953 on topoisomerase II-mediated DNA cleavage was assessed using pBR322 DNA as a substrate (Figure 4B). Use of this plasmid allowed quinolone effects to be monitored at a broad spectrum of sites and at drug concentrations (5−100 μM) lower than those that could be used with the oligonucleotide substrate. If the presence of quinolone on only one DNA strand is sufficient to generate double-stranded breaks, then double-stranded breaks should be observed even at very low drug concentrations. Conversely, if quinolone occupancy on both DNA strands is required, single-stranded breaks should predominate at low drug concentrations and should be converted to double-stranded breaks only at higher quinolone levels.

The plasmid study clearly supports the hypothesis that the action of CP-115,953 on only one DNA strand is sufficient to stimulate topoisomerase II-mediated double-stranded cleavage. Over the quinolone range that was employed, levels of single-stranded DNA breaks never increased by more than 1.5-fold. In contrast, levels of double-stranded DNA breaks increased ∼4-fold at the lowest quinolone concentration that was examined (5 μM) and rose steadily up to ∼20-fold at 100 μM CP-115,953.

A Model for the Stimulation of Topoisomerase II-Mediated Double-Stranded DNA Breaks by Quinolones. The data presented above are consistent with the following model for quinolone action against human topoisomerase IIα (Figure 5). The proposed mechanism of drug action is adapted from a model previously described for the stimulation of topoisomerase II-mediated DNA cleavage by DNA lesions (*13*, *32*, *36*).

As depicted in Figure 5, the interaction of quinolone at either scissile bond of a cleavage site perturbs the structure of the double helix (*48*−*50*). This distortion, in turn, creates a preferred substrate for DNA scission that is recognized by both protomer active sites of topoisomerase II. As a result, the presence of quinolone on only one strand of the genetic material is sufficient to increase the rate of enzyme-mediated scission on both strands and to generate a double-stranded DNA break.

An alternative interpretation of the data is that two quinolone molecules (one on each strand) are necessary to promote double-stranded DNA cleavage; however, the binding of CP-115,953 to one scissile bond rapidly promotes the binding of a second drug molecule to the scissile bond on the opposite strand (*52*, *53*). We believe that this latter interpretation is not correct for the following reason. In the oligonucleotide cleavage experiments, quinolone saturation was observed at ∼100 μM drug. If the "cooperative drug binding model" were applicable, substrates that contained a strong quinolone site on one DNA strand would contain drug molecules on both strands at this saturating concentration. Consequently, the introduction of a second strong quinolone site on the opposite strand of the substrate would have no further effect on the maximal level of DNA cleavage. As seen Figures 3 and 4, this was not the case. The introduction of a second strong quinolone site doubled the maximal level of DNA cleavage induced by CP-115,953.

This study further highlights differences between drugs that stimulate the forward rate of topoisomerase II-mediated DNA cleavage versus those that inhibit the ligation reaction of the enzyme. Results suggest that drugs in the former group may be more likely to generate enzyme-linked double-stranded DNA breaks than those in the latter group (*34*). This finding has potentially significant implications for the mechanism by which transient topoisomerase II−DNA cleavage complexes are converted to permanent DNA breaks in the cell. Furthermore, it implies that there may be subtle, but important, differences in the pathways that are used to repair the genomic damage that is generated by these drugs.

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REFERENCES

1. Wang, J. C. (1996) *Annu. Rev. Biochem. 65*, 635−692.
2. Nitiss, J. L. (1998) *Biochim. Biophys. Acta 1400*, 63−81.
3. Burden, D. A., and Osheroff, N. (1998) *Biochim. Biophys. Acta 1400*, 139−154.
4. Fortune, J. M., and Osheroff, N. (2000) *Prog. Nucleic Acid Res. Mol. Biol. 64*, 221−253.
5. Champoux, J. J. (2001) *Annu. Rev. Biochem. 70*, 369−413.
6. Sabourin, M., and Osheroff, N. (2002) in *Encyclopedia of Molecular Medicine* (Creighton, T. E., Ed.) pp 3192−3197, John Wiley & Sons, Inc., New York.
7. Wang, J. C. (2002) *Nat. Rev. Mol. Cell Biol. 3*, 430−440.
8. Berger, J. M. (1998) *Biochim. Biophys. Acta 1400*, 3−18.
9. Wang, J. C. (1998) *Q. Rev. Biophys. 31*, 107−144.
10. Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M., and Chen, G. L. (1983) *J. Biol. Chem. 258*, 15365−15370.
11. Sander, M., and Hsieh, T. (1983) *J. Biol. Chem. 258*, 8421−8428.
12. Zechiedrich, E. L., Christiansen, K., Andersen, A. H., Westergaard, O., and Osheroff, N. (1989) *Biochemistry 28*, 6229−6236.
13. Wilstermann, A. M., and Osheroff, N. (2002) *Curr. Top. Med. Chem. 3*, 1349−1364.
14. Howard, M. T., Neece, S. H., Matson, S. W., and Kreuzer, K. N. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 12031−12035.
15. Li, T. K., and Liu, L. F. (2001) *Annu. Rev. Pharmacol. Toxicol. 41*, 53−77.
16. Kaufmann, S. H. (1998) *Biochim. Biophys. Acta 1400*, 195−211.
17. Baguley, B. C., and Ferguson, L. R. (1998) *Biochim. Biophys. Acta 1400*, 213−222.
18. Felix, C. A. (1998) *Biochim. Biophys. Acta 1400*, 233−255.
19. Rowley, J. D. (1998) *Annu. Rev. Genet. 32*, 495−519.
20. Walker, J. V., and Nitiss, J. L. (2002) *Cancer Invest. 20*, 570−589.
21. Pommier, Y., Fesen, M. R., and Goldwasser, F. (1996) in *Cancer Chemotherapy and Biotherapy*: *Principles and Practice* (Chabner, B. A., and Longo, D. L., Eds.) pp 435−461, Lippincott-Raven Publishers, Philadelphia.
22. Pommier, Y. (1997) in *Cancer Therapeutics*: *Experimental and Clinical Agents* (Teicher, B. A., Ed.) pp 153−174, Humana Press, Totowa, NJ.
23. Kreuzer, K. N., and Cozzarelli, N. R. (1979) *J. Bacteriol. 140*, 424−435.
24. Andoh, T., and Ishida, R. (1998) *Biochim. Biophys. Acta 1400*, 155−171.
25. Osheroff, N. (1989) *Biochemistry 28*, 6157−6160.
26. Robinson, M. J., and Osheroff, N. (1990) *Biochemistry 29*, 2511−2515.
27. Byl, J. A. W., Fortune, J. M., Burden, D. A., Nitiss, J. L., and Osheroff, N. (1999) *Biochemistry 38*, 15573−15579.
28. van Hille, B., Perrin, D., and Hill, B. T. (1999) *Anticancer Drugs 10*, 551−560.
29. Robinson, M. J., Martin, B. A., Gootz, T. D., McGuirk, P. R., Moynihan, M., Sutcliffe, J. A., and Osheroff, N. (1991) *J. Biol. Chem. 266*, 14585−14592.
30. Froelich-Ammon, S. J., Patchan, M. W., Osheroff, N., and Thompson, R. B. (1995) *J. Biol. Chem. 270*, 14998−15005.
31. Kingma, P. S., Corbett, A. H., Burcham, P. C., Marnett, L. J., and Osheroff, N. (1995) *J. Biol. Chem. 270*, 21441−21444.
32. Kingma, P. S., and Osheroff, N. (1997) *J. Biol. Chem. 272*, 1148−1155.
33. Sabourin, M., and Osheroff, N. (2000) *Nucleic Acids Res. 28*, 1947−1954.
34. Bromberg, K. D., Burgin, A. B., and Osheroff, N. (2003) *J. Biol. Chem. 278*, 7406−7412.
35. Kingma, P. S., and Osheroff, N. (1997) *J. Biol. Chem. 272*, 7488−7493.
36. Kingma, P. S., and Osheroff, N. (1998) *Biochim. Biophys. Acta 1400*, 223−232.
37. Wasserman, R. A., Austin, C. A., Fisher, L. M., and Wang, J. C. (1993) *Cancer Res. 53*, 3591−3596.
38. Kingma, P. S., Greider, C. A., and Osheroff, N. (1997) *Biochemistry 36*, 5934−5939.
39. Bromberg, K. D., Hendricks, C., Burgin, A. B., and Osheroff, N. (2002) *J. Biol. Chem. 277*, 31201−31206.
40. Wilstermann, A. M., and Osheroff, N. (2001) *J. Biol. Chem. 276*, 17727−17731.
41. Bromberg, K. D., and Osheroff, N. (2001) *Biochemistry 40*, 8410−8418.
42. Robinson, M. J., Martin, B. A., Gootz, T. D., McGuirk, P. R., and Osheroff, N. (1992) *Antimicrob. Agents Chemother. 36*, 751−756.
43. Anderson, V. E., Zaniewski, R. P., Kaczmarek, F. S., Gootz, T. D., and Osheroff, N. (1999) *J. Biol. Chem. 274*, 35927−35932.
44. Anderson, V. E., Zaniewski, R. P., Kaczmarek, F. S., Gootz, T. D., and Osheroff, N. (2000) *Biochemistry 39*, 2726−2732.
45. Corbett, A. H., Zechiedrich, E. L., and Osheroff, N. (1992) *J. Biol. Chem. 267*, 683−686.
46. Spitzner, J. R., Chung, I. K., Gootz, T. D., McGuirk, P. R., and Muller, M. T. (1995) *Mol. Pharmacol. 48*, 238−249.
47. Strumberg, D., Nitiss, J. L., Dong, J., Kohn, K. W., and Pommier, Y. (1999) *J. Biol. Chem. 274*, 28246−28255.
48. Marians, K. J., and Hiasa, H. (1997) *J. Biol. Chem. 272*, 9401−9409.
49. Kwok, Y., and Hurley, L. H. (1998) *J. Biol. Chem. 273*, 33020−33026.
50. Kwok, Y., Zeng, Q., and Hurley, L. H. (1999) *J. Biol. Chem. 274*, 17226−17235.
51. Kingma, P. S., and Osheroff, N. (1998) *J. Biol. Chem. 273*, 17999−18002.
52. Shen, L. L., Baranowski, J., and Pernet, A. G. (1989) *Biochemistry 28*, 3879−3885.
53. Shen, L. L., Mitscher, L. A., Sharma, P. N., O'Donnell, T. J., Chu, D. W., Cooper, C. S., Rosen, T., and Pernet, A. G. (1989) *Biochemistry 28*, 3886−3894.

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