

Quinone-Induced Enhancement of DNA Cleavage by Human Topoisomerase II α : Adduction of Cysteine Residues 392 and 405[†]

Ryan P. Bender,[‡] Amy-Joan L. Ham,^{‡,§} and Neil Osheroff^{*,‡,||}

Departments of Biochemistry and Medicine (Hematology/Oncology) and Mass Spectrometry Research Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

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ABSTRACT: Several quinone-based metabolites of drugs and environmental toxins are potent topoisomerase II poisons. These compounds act by adducting the protein and appear to increase levels of enzyme–DNA cleavage complexes by at least two potentially independent mechanisms. Treatment of topoisomerase II α with quinones inhibits DNA religation and blocks the N-terminal gate of the protein by cross-linking its two protomer subunits. It is not known whether these two effects result from adduction of quinone to the same amino acid residue(s) in topoisomerase II α or whether they are mediated by modification of separate residues. Therefore, this study identified amino acid residues in human topoisomerase II α that are modified by quinones and determined their role in the actions of these compounds as topoisomerase II poisons. Four cysteine residues were identified by mass spectrometry as sites of quinone adduction: Cys170, Cys392, Cys405, and Cys455. Mutations (Cys \rightarrow Ala) were individually generated at each position. Only mutations at Cys392 or Cys405 reduced sensitivity (\sim 50% resistance) to benzoquinone. Top2 α C392A and top2 α C405A displayed faster rates (\sim 2-fold) of DNA religation than wild-type topoisomerase II α in the presence of the quinone. In contrast, as determined by DNA binding, protein clamp closing, and protomer cross-linking experiments, mutations at Cys392 and Cys405 did not affect the ability of benzoquinone to block the N-terminal gate of topoisomerase II α . These findings indicate that adduction of Cys392 and Cys405 is important for the actions of quinones against the enzyme and increases levels of cleavage complexes primarily by inhibiting DNA religation.

Topoisomerase II α is an essential enzyme that plays important roles in a number of growth-related processes in human cells, including DNA replication and chromosome segregation. The enzyme removes superhelical twists, knots, and tangles from the genetic material by generating transient double-stranded breaks in the backbone of DNA (1–7). To maintain genomic integrity during this cleavage event, the enzyme forms covalent bonds between active site tyrosyl residues and the 5'-DNA termini created by scission of the double helix (8–10). Although these transient covalent topoisomerase II α -cleaved DNA complexes (i.e., cleavage complexes) are requisite intermediates in the catalytic cycle of the enzyme, their presence is potentially deleterious to the cell. When a DNA tracking system, such as a replication fork, attempts to traverse a cleavage complex, the transient enzyme-bound DNA break often is converted to a permanent strand break (1–4, 7). Thus, conditions that significantly increase the concentration of topoisomerase II α –DNA cleavage complexes trigger illegitimate recombination, chromosomal aberrations, and cell death pathways (4, 11–16).

Agents that increase levels of topoisomerase II α -mediated DNA strand breaks are termed topoisomerase II poisons to distinguish their mechanism of action from that of compounds that act by inhibiting the overall catalytic activity of the enzyme (i.e., catalytic inhibitors) (4, 15, 17–19). Topoisomerase II poisons play important roles in both the treatment and the development of human cancers. Approximately one-half of all cancer chemotherapy regimens contain drugs that target the type II enzyme. Furthermore, several topoisomerase II poisons that are naturally found in fruits and vegetables, such as bioflavonoids, are believed to have chemopreventative properties (20–23). Conversely, a small number of patients who receive therapy that includes topoisomerase II-targeted drugs develop secondary leukemias. These drug-related leukemias commonly feature aberrations (deletions, duplications, or translocations) in the mixed-lineage leukemia (*MLL*) gene at chromosomal band 11q23 (13, 14, 24–29). In addition, the same bioflavonoids that are beneficial for adult health, when ingested during pregnancy, are believed to increase the risk of infant leukemias that include *MLL* rearrangements (30–34).

Recently, a number of toxic quinone-based metabolites of drugs or environmental pollutants have been found to be potent human topoisomerase II poisons (35). These include *N*-acetyl-*p*-benzoquinone imine (derived from acetaminophen) (36), benzoquinone (derived from benzene) (37, 38),

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* To whom correspondence should be addressed. Telephone: (615) 322-4338. Fax: (615) 343-1166. E-mail: neil.osheroff@vanderbilt.edu.

[‡] Department of Biochemistry.

[§] Mass Spectrometry Research Center.

^{||} Department of Medicine (Hematology/Oncology).

and several polychlorinated biphenyl (PCB)¹ metabolites (39). Exposure to these compounds causes DNA strand breaks and other chromosomal aberrations and has been linked to a variety of human health problems, including cancer (40–44). To this point, the accumulation of benzoquinone in the bone marrow is believed to contribute to the leukemogenic properties of benzene (41). Moreover, genetic deficiencies in NAD(P)H:quinone oxidoreductase 1, the enzyme that reduces benzoquinone to the less reactive hydroquinone, are linked to the formation of leukemias with *MLL* rearrangements (45–49).

The quinone-based topoisomerase II poisons differ from “classic” topoisomerase II poisons (such as etoposide and bioflavonoids) in two ways. First, quinones are mixed-function inhibitors of the type II enzyme: they increase DNA scission when added to a topoisomerase II–DNA complex but inhibit scission when incubated with the enzyme prior to the addition of nucleic acids (36–39, 50–54). Second, in contrast to compounds like etoposide (which interact with topoisomerase II in a noncovalent manner), quinones act by covalently attaching to the protein (35–39, 53).

Because of the human health concerns of quinone-based topoisomerase II poisons and their unusual mode of action against the type II enzyme, sites of quinone adduction on human topoisomerase II α were mapped by mass spectrometry. Four cysteine residues were identified and mutated to alanine residues. Two of the mutant enzymes, top2 α C392A and top2 α C405A, exhibited decreased sensitivity to benzoquinone and the PCB quinone 4'Cl-2,5pQ. These findings strongly suggest that adduction of cysteine residues 392 and 405 plays a role in the actions of quinones against human topoisomerase II α .

EXPERIMENTAL PROCEDURES

Enzymes and Materials. Human topoisomerase II α was expressed in *Saccharomyces cerevisiae* and purified as described previously (55–57). Mass spectrometry grade trypsin and chymotrypsin were obtained from Fisher and Princeton Separations, respectively. Negatively supercoiled pBR322 DNA was prepared using a Plasmid Mega Kit (Qiagen) as described by the manufacturer. Benzoquinone, plumbagin, and etoposide were obtained from Sigma, prepared as 20 mM stock solutions in 100% DMSO, and stored at 4 °C. 4'Cl-2,5pQ (the generous gift of H. J. Lehmler, University of Iowa, Iowa City, IA) was synthesized by coupling 4'-chloroaniline with 1,4-benzoquinone (58). The compound was prepared as a 20 mM stock in 100% DMSO and stored at –20 °C. All other chemicals were analytical reagent grade.

Adduction of Topoisomerase II α by Quinones. To identify sites of quinone adduction on human topoisomerase II α , 135 nM enzyme was incubated in a total of 300 μ L of cleavage buffer [10 mM Tris-HCl (pH 7.9), 100 mM KCl, 5 mM MgCl₂, 0.1 mM NaEDTA, and 2.5% glycerol] in the presence of 250 μ M plumbagin for 10 min at 37 °C. Reactions were quenched with 20 μ L of 45 mM DTT. Samples were applied to 30 000 NMWL filter columns (Millipore), washed with 10% methanol in 25 mM am-

monium bicarbonate, and resuspended in 25 μ L of 25 mM ammonium bicarbonate. DTT (2.5 μ L of a 45 mM solution) was added, and the protein was incubated for 15 min at 50 °C. Iodoacetamide (2.5 μ L of a 100 mM solution) was added, and samples were incubated at room temperature in the absence of light for 15 min. Additional DTT (2.5 μ L of a 45 mM solution) was added to quench the iodoacetamide, and the protein was incubated for 15 min at 50 °C. Samples were washed with 10% methanol in 25 mM ammonium bicarbonate and resuspended in 25 μ L of 25 mM ammonium bicarbonate. Trypsin (0.1 μ g) was added, and samples were incubated for 16 h at 37 °C to digest the protein. Alternatively, chymotrypsin (0.1 μ g) was added, and samples were incubated for 6 h at room temperature. Peptides were eluted from the column, dried, and reconstituted in 0.1% formic acid for mass spectral analysis.

Residues in topoisomerase II α that were adducted by quinone were identified by mass spectral analysis of the tryptic or chymotryptic peptides. The LC–MS/MS analyses were performed on a ThermoFinnigan LTQ linear ion trap mass spectrometer equipped with a ThermoFinnigan Surveyor LC pump and autosampler, NanoSpray source (Thermo Electron), and Xcalibur 1.4 instrument control and data analysis software. HPLC separation of the tryptic peptides was conducted using a 100 μ m \times 11 cm C-18 capillary column (Monitor C18, 5 μ m, 100 Å, Column Engineering) at a flow rate of 0.7 μ L/min. Solvent A was H₂O with 0.1% formic acid, and solvent B was acetonitrile containing 0.1% formic acid. The gradient program was as follows: linear gradient from 0 to 5% B from 0 to 3 min, 5% B from 3 to 5 min, linear gradient to 50% B from 5 to 50 min, linear gradient to 80% B from 50 to 52 min, linear gradient to 90% B from 52 to 55 min, and 90% B in solvent A from 55 to 56 min. MS/MS scans were acquired using an isolation width of *m/z* 2, an activation time of 30 ms, an activation *Q* of 0.250, and 30% normalized collision energy using one microscan and an ion time of 100 for each MS/MS scan. The mass spectrometer was tuned prior to analysis using the synthetic peptide TpepK (AVAGKAGAR). Some parameters may have varied slightly from experiment to experiment, but typically, the tune parameters were as follows: spray voltage of 2.0 kV, capillary temperature of 160 °C, capillary voltage of 60 V, and tube lens of 130 V. Tandem MS analysis was performed using data-dependent scanning in which one full MS spectrum, using a full mass range of 400–2000 amu, was followed via three MS/MS spectra. Wild-type and modified peptides were identified using the SEQUEST algorithm (59) and the SEQUEST Browser software (Thermo Electron, San Jose, CA) using the human subset of the NCBI database. In addition, lists of theoretical or SEQUEST-identified unmodified peptides were created, and each peptide was run through P-Mod software to check for possible chemical modifications (60). The candidate modifications found by the software were verified by visual inspection of corresponding spectra.

Generation of Mutant Human Topoisomerase II α Proteins. Mutations in the topoisomerase II α PCR substrate were generated by cloning a Sall-KpnI fragment of YEpWob6 (61) that encoded the N-terminus of the human enzyme into pUC18. Site-directed mutagenesis was performed using the QuickChange II PCR system (Stratagene). The sequences

¹ Abbreviations: PCB, polychlorinated biphenyl; 4'Cl-2,5pQ, 2-(4-chlorophenyl)[1,4]benzoquinone.

of the forward and reverse primers used to generate the C170A mutation were GGAGCCAAATTGGCTAACATATTCAGTACCAAATTTACTGTGG and GAATATGTTAGCCAATTTGGCTCCATAGCCATTTTCGACCACC, respectively. The sequences of the forward and reverse primers used to generate the C392A mutation were CTTTACAACCCAAGAGCTTTGGATCAACAGCCCAATTGAGTG and GATAAATTTTTCCTCAATTGGGCTGTTGATCCAAAGCTCTTGG, respectively. The sequences of the forward and reverse primers used to generate the C405A mutation were CAAAGCTGCCATTGGCGCTGGTATTGTAGAAAGCATAC and CAGTTTAGTATGCTTTC-TACAATACCAGCGCCAATGGCAGC, respectively. The mutagenized codons are underlined. To generate the C392/405A double mutation, sequential rounds of PCR were performed using the primers to generate the C392A and C405A mutations as described above. Mutations were verified by sequencing, and SalI–KpnI fragments were cloned back into YEpWob6. Mutant human topoisomerase II α enzymes were purified as described above.

Site-Specific DNA Cleavage. DNA sites cleaved by human topoisomerase II α were identified as described by O'Reilly and Kreuzer (62). A linear 4330 bp fragment (HindIII–EcoRI) of pBR322 plasmid DNA singly labeled with 32 P on the 5'-terminus of the HindIII site was used as the cleavage substrate. Reaction mixtures contained 0.35 nM DNA substrate and 60 nM human topoisomerase II α in 50 μ L of cleavage buffer containing 1 mM ATP. Assays were carried out in the absence of drug or in the presence of 12.5 μ M etoposide, 25 μ M benzoquinone, or 100–500 μ M plumbagin. Reaction mixtures were incubated for 10 min at 37 °C. Cleavage intermediates were trapped by adding 5 μ L of 5% SDS followed by 3 μ L of 375 mM NaEDTA (pH 8.0). Topoisomerase II α was digested with proteinase K (5 μ L of a 0.8 mg/mL solution) for 30 min at 45 °C. Reaction products were precipitated twice in ethanol, dried, and resuspended in 40% formamide, 8.4 mM EDTA, 0.02% bromophenol blue, and 0.02% xylene cyanol FF. Samples were subjected to electrophoresis in a 6% sequencing gel. The gel was then fixed in a 10% methanol/10% acetic acid mixture for 5 min and dried, and DNA cleavage products were analyzed on a Bio-Rad FX Molecular Imager.

Cleavage of Plasmid DNA. DNA cleavage reactions were carried out using the procedure of Fortune and Osheroff (63). Unless stated otherwise, assay mixtures contained 135 nM wild-type or mutant topoisomerase II α and 10 nM negatively supercoiled pBR322 DNA in a total of 20 μ L of cleavage buffer that contained 0–200 μ M benzoquinone, 4'Cl-2,5pQ, or etoposide. DNA cleavage was initiated by shifting mixtures to 37 °C, and samples were incubated for 6 min to establish DNA cleavage–religation equilibria. Enzyme–DNA cleavage intermediates were trapped by adding 2 μ L of 5% SDS and 1 μ L of 375 mM EDTA (pH 8.0). Proteinase K was added (2 μ L of a 0.8 mg/mL solution), and reaction mixtures were incubated for 30 min at 45 °C to digest the topoisomerase II α . Samples were mixed with 2 μ L of 60% sucrose in 10 mM Tris-HCl (pH 7.9), 0.5% bromophenol blue, and 0.5% xylene cyanol FF, heated for 15 min at 45 °C, and subjected to electrophoresis in 1% agarose gels in 40 mM Tris-acetate (pH 8.3) and 2 mM EDTA that contained 0.5 μ g/mL ethidium bromide. DNA cleavage was monitored by the conversion of negatively supercoiled plasmid DNA

to linear molecules. DNA bands were visualized by ultraviolet light and quantified using an Alpha Innotech digital imaging system.

DNA Religation. The DNA religation reaction of human topoisomerase II α was monitored according to the procedure of Byl et al. (64). Topoisomerase II α DNA cleavage–religation equilibria were established using a plasmid substrate as described in the preceding section in the absence or presence of 100 μ M benzoquinone. Religation was initiated by shifting reaction mixtures from 37 to 0 °C, and reactions were stopped by the addition of 2 μ L of 5% SDS followed by 1 μ L of 375 mM NaEDTA (pH 8.0). Samples were processed and analyzed as described above for topoisomerase II α cleavage reactions.

DNA Binding. Binding of topoisomerase II α to linear DNA substrates was assessed using a nitrocellulose filter binding assay. Either linearized pBR322 DNA (described above) or a 50-mer oligonucleotide was used. The 50-mer oligonucleotide containing a single topoisomerase II cleavage site and its complementary strand were prepared on an Applied Biosystems DNA synthesizer. The sequences of the top and bottom strands were 5'-TTGGTATCTGCGCTCTGCTGAAGCC↓AGTTACCTTCGGAAAAAGAGTTGGT-3' and 5'-ACCAACTCTTTTCCGAAGGT↓AACTGGCTTCAGCAGAGCGCAGATACCAA-3', respectively. This substrate contains a single topoisomerase II cleavage site (65) that is denoted by the arrows. Wild-type or mutant topoisomerase II α was incubated with benzoquinone for 5 min at 37 °C in 15 μ L of DNA binding buffer [10 mM Tris-HCl (pH 7.9), 30 mM KCl, 0.1 mM NaEDTA, and 2.5% glycerol]. Binding was initiated by adding DNA in 5 μ L of binding buffer, and samples were incubated at 37 °C for 6 min. The final concentrations of topoisomerase II α , DNA, and benzoquinone in the binding mixtures were 400 nM, 5 nM, and 100 μ M, respectively. Nitrocellulose membranes (0.45 μ m HA, Millipore) were prepared by incubation in DNA binding buffer for 10 min. Samples were applied to the membranes and filtered in vacuo. Membranes were washed three times with 1 mL of DNA binding buffer, dried, and submerged in 8 mL of scintillation fluid (Econo-Safe, Research Products International). The radioactivity remaining on the membranes was quantified using a Beckman LS 5000 TD scintillation counter.

Assays that monitored binding to a plasmid substrate were carried out according to the procedure of Fortune and Osheroff (66). Reaction mixtures contained 400 nM enzyme and 100 μ M benzoquinone in 15 μ L of binding buffer. Samples were incubated for 5 min at 37 °C. Binding was initiated by adding DNA in 5 μ L of binding buffer, and samples were incubated for 6 min at 37 °C. The concentrations of topoisomerase II α , DNA, and benzoquinone in the final mixtures were 400 nM, 5 nM, and 100 μ M, respectively. Samples were loaded directly onto a 1% agarose gel and subjected to electrophoresis in 100 mM Tris-borate (pH 8.3) and 2 mM EDTA. Gels were stained for 30 min with 0.5 μ g/mL ethidium bromide, and samples were analyzed as described above.

Protein Clamp Closing. Filter binding assays were used to analyze the salt-stable closed clamp of topoisomerase II α (39, 67). Briefly, 5 nM wild-type or mutant human topoisomerase II α and 2 nM pBR322 were incubated for 4 min

at 37 °C in a total of 90 μ L of clamp closing buffer [50 mM Tris-HCl (pH 8.0), 100 mM KCl, 1 mM EDTA, and 8 mM MgCl_2]. Benzoquinone (10 μ L of a 1 mM solution in 10% DMSO) or an equivalent amount of solvent was added, and mixtures were incubated for an additional 6 min at 37 °C.

Binding mixtures were loaded onto glass fiber filters (Millipore) preincubated in clamp closing buffer and filtered in vacuo. Filters were washed three times with clamp closing buffer (low-salt wash), followed by three washes with clamp closing buffer that contained 1 M NaCl (high-salt wash), and three washes with 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 0.5% SDS. DNA was precipitated with 2-propanol and loaded onto a 1% agarose gel in 40 mM Tris-acetate (pH 8.3) and 2 mM EDTA that contained 0.5 μ g/mL ethidium bromide. DNA was visualized as described above.

Protein Cross-Linking. The ability of quinones to cross-link the protomer subunits of human topoisomerase II α was assessed using SDS–polyacrylamide gels. Cross-linking mixtures contained 135 nM wild-type or mutant topoisomerase II α in a total of 60 μ L of cleavage buffer. The enzyme was incubated for 0–4 min at 37 °C in the presence of 100 μ M benzoquinone. Reactions were quenched by the addition of 6 μ L of 5 mM DTT followed by an additional 2 min incubation at 37 °C. Samples were precipitated with TCA and resuspended in 10 μ L of H_2O . Laemmli buffer (10 μ L, Bio-Rad) was added, and samples were subjected to electrophoresis in a 7.5% denaturing acrylamide gel (Bio-Rad) at 200 V for 1 h. The protein was visualized by Coomassie staining.

RESULTS

Identification of Cysteine Residues in Human Topoisomerase II α that Are Adducted by Quinones. Quinones are unique among topoisomerase II poisons in that their actions require adduction to the enzyme (35–39, 53). It is not known how this covalent modification increases levels of cleavage complexes, and it is possible that quinones affect enzyme activity by more than one process. Religation experiments suggest that these compounds function at least in part by impairing the ability of topoisomerase II to rejoin cleaved DNA termini (36–39). In addition, quinones block the N-terminal gate of the protein, thereby increasing the concentration of DNA associated with topoisomerase II by trapping the substrate in a noncovalent complex (39). Molecular sizing experiments suggest that quinones block the N-terminal gate by cross-linking the two protomer subunits of topoisomerase II α (39).

In an effort to further dissect the mechanistic basis for the actions of quinones as topoisomerase II poisons, amino acid residues in human topoisomerase II α that are covalently modified by these compounds were identified by mass spectrometry. Initial attempts utilized benzoquinone as the modification agent. Unfortunately, no individual amino acid residues were identified in these experiments. This is most likely due to the ability of benzoquinone to cross-link multiple residues in the protein (53).

To overcome this technical difficulty, plumbagin, which has only a single reactive site, was employed (Figure 1). Plumbagin is a topoisomerase II poison; however, it is at least 10-fold less potent than benzoquinone (Figure 1). In

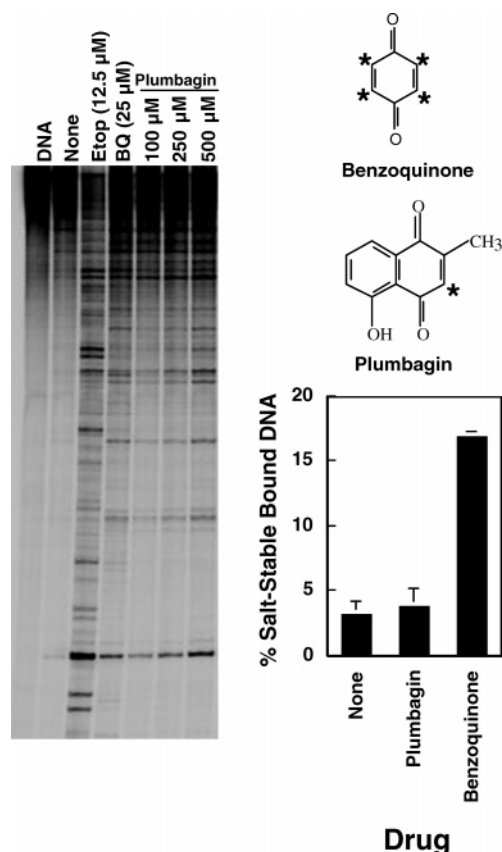


FIGURE 1: Enhancement of DNA cleavage by human topoisomerase II α in the presence of plumbagin. The structures of plumbagin and benzoquinone are shown. Reactive sites on the compounds are denoted with asterisks. An autoradiogram of a polyacrylamide gel of DNA cleavage reactions is shown. Assays contained no compound (None), 12.5 μ M etoposide, 25 μ M benzoquinone, or 100–500 μ M plumbagin. A DNA control is shown in the far left lane. Data are representative of two independent experiments. Results of topoisomerase II protein clamp closing assays are shown as a bar graph. The graph represents levels of salt-stable bound DNA formed when topoisomerase II–DNA complexes were treated with no drug (None), 250 μ M plumbagin, or 100 μ M benzoquinone. Error bars represent the standard deviation of four independent experiments.

addition, the compound does not effectively block the N-terminal gate of the enzyme (Figure 1) and does not cross-link the protomer subunits (39). These data support the hypothesis that multiple processes contribute to the increase in cleavage complexes by quinone-based topoisomerase II poisons.

As determined by LC–MS/MS analysis of tryptic and chymotryptic peptides derived from human topoisomerase II α , four cysteine residues were adducted by plumbagin, Cys170, Cys392, Cys405, and Cys455.² Representative spectra are shown in Figure S1A–D of the Supporting

² Human topoisomerase II α contains 13 cysteine residues. Two of these residues, Cys300 and Cys862, were not covered by the digests used in this study. The N-terminal peptide also was not identified by mass spectral analysis. Adducted Cys170 and Cys392 were identified in two independent tryptic digests of topoisomerase II α . Adducted Cys455 was identified in one of the tryptic digests and two independent chymotryptic digests. Adducted Cys405 was identified in only one of the chymotryptic digests. However, since this latter residue is known to exist as a free sulfhydryl in the protein and is solvent accessible, the decision was made to continue studies with this residue as a site of quinone modification.

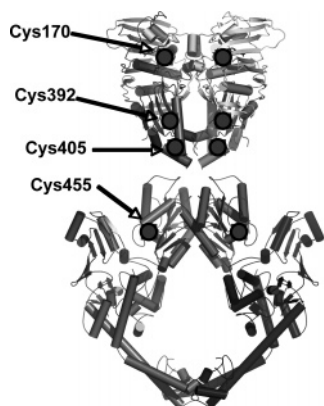


FIGURE 2: Plumbagin adducts to cysteine residues in human topoisomerase II α . Residues adducted by quinones were identified by mass spectrometry. A composite of the crystal structures of the yeast catalytic core and N-terminal domain is shown, and the locations of the homologous cysteine residues adducted in human topoisomerase II α are denoted with dark gray circles. These residues were mutated to alanines using mutagenesis PCR to evaluate their role in quinone action. This figure was adapted from refs 68 and 69.

Information. Modeling studies based on the structure of yeast topoisomerase II place Cys170, Cys392, Cys405 in the N-terminal domain and Cys455 in the B' subdomain of the catalytic core of the enzyme (68, 69) (Figure 2). As determined in a previous study, all of these residues exist in the protein as free sulfhydryls (i.e., not part of a disulfide bridge) with the exception of Cys455, which is free only 10–15% of the time (70).

DNA Cleavage Mediated by Mutant Human Topoisomerase II α Enzymes. To assess the contributions of the four cysteine residues listed above to the actions of quinones as topoisomerase II poisons, each of the residues was individually mutated to an alanine residue, and the resulting mutant enzymes (top2 α C170A, top2 α C392A, top2 α C405A, and top2 α C455A, respectively) were purified and characterized. With the exception of top2 α C455A, all of the mutant enzymes exhibited wild-type DNA cleavage activity in the absence of topoisomerase II poisons (Figure 3A). Top2 α C455A exhibited a cleavage activity that was ~50% higher than that of the wild type (not shown). Since the basal properties of this mutant enzyme were altered, it was not utilized further in this study. It should be noted, however, that top2 α C455A exhibited no resistance toward quinones (not shown).

To further analyze the properties of top2 α C170A, top2 α C392A, and top2 α C405A, the sensitivity of these mutant enzymes to topoisomerase II poisons was determined. Etoposide, a classic topoisomerase II poison, and benzoquinone and the PCB metabolite 4'Cl-2,5pQ, two quinone-based topoisomerase II poisons, were utilized for these experiments. As shown in Figure 3B, all displayed wild-type sensitivity to the anticancer drug etoposide. In contrast, two of the mutant enzymes, top2 α C392A and top2 α C405A, were less responsive to benzoquinone (Figure 3C) and 4'Cl-2,5pQ (Figure 3D) than was wild-type topoisomerase II α . The decreased sensitivity did not appear to be due to a reduced affinity for quinone, because at saturating levels of benzoquinone or 4'Cl-2,5pQ, levels of DNA cleaved by the mutant enzymes were only one-half of those observed with the parental enzyme.

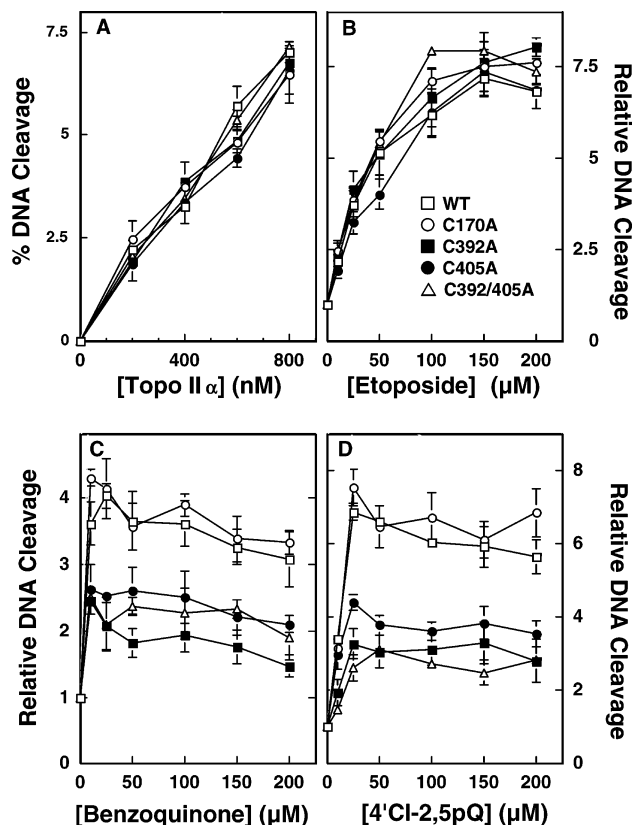


FIGURE 3: DNA cleavage activity of mutant human topoisomerase II α enzymes. (A) Cleavage activity was assessed using 0–800 nM topoisomerase II α in the absence of quinones. Assay mixtures contained wild-type enzyme (\square), top2 α C170A (\circ), top2 α C392A (\blacksquare), top2 α C405A (\bullet), or top2 α C392/405A (\triangle). (B) DNA cleavage reactions were carried out in the presence of 0–200 μ M etoposide. (C) DNA cleavage reactions were carried out in the presence of 0–200 μ M benzoquinone. (D) DNA cleavage reactions were carried out in the presence of 0–200 μ M 4'Cl-2,5pQ. Error bars represent the standard deviation of at least three independent experiments.

The finding that mutation of Cys392 or Cys405 causes partial resistance toward quinones can be explained by two possibilities. For example, one residue may be involved in the inhibition of DNA religation, while the other may be involved in blocking the N-terminal gate. If this were the case, an enzyme that carries alanine residues at both positions (top2 α C392/405A) should display enhanced resistance over either singly mutated enzyme.

Alternatively, adduction of these residues by quinones may enhance topoisomerase II α -mediated DNA cleavage by the same mechanism (religation or the N-terminal gate). However, it is necessary to modify both residues to observe an effect. If this were the case, the doubly mutated enzyme should display a sensitivity to quinones that is similar to that of either singly mutated enzyme.

To address this mechanistic issue, top2 α C392/405A was generated and isolated. Like top2 α C392A and top2 α C405A, the doubly mutated enzyme exhibited wild-type DNA cleavage activity in the absence (Figure 3A) or presence (Figure 3B) of etoposide but displayed approximately one-half of the sensitivity to either benzoquinone (Figure 3C) or 4'Cl-2,5pQ (Figure 3D). These findings suggest that quinone adduction of Cys392 and Cys405 enhances topoisomerase II α -mediated DNA cleavage as part of the same process.

Finally, two additional cysteine residues, Cys216 and Cys300, have been shown to exist in the protein as free

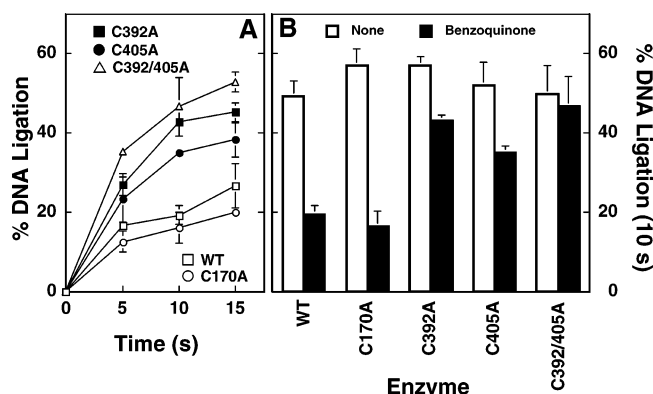


FIGURE 4: Quinone-resistant mutant human topoisomerase II α enzymes display higher rates of DNA religation in the presence of benzoquinone. (A) DNA religation reactions were carried out in the presence of 100 μ M benzoquinone. Assay mixtures contained wild-type enzyme (\square), top2 α C170A (\circ), top2 α C392A (\blacksquare), top2 α C405A (\bullet), or top2 α C392/405A (\blacktriangle). Samples were incubated at 37 $^{\circ}$ C to establish DNA cleavage–religation equilibria. Reaction mixtures were shifted to 0 $^{\circ}$ C to initiate religation. DNA religation was quantified by the loss of linear cleaved molecules. (B) Representative DNA religation data at 10 s. DNA cleavage–religation equilibria were established in the presence (black bars) or absence (white bars) of benzoquinone. Error bars represent the standard deviation of at least three independent experiments.

sulfhydryls (70). Even though they were not identified as sites of quinone adduction in our work, top2 α C216A and top2 α C300A were generated and analyzed. Neither enzyme exhibited any resistance to benzoquinone or 4'Cl-2,5pQ (not shown).

Effects of Benzoquinone on DNA Religation Mediated by Mutant Human Topoisomerase II α Enzymes. To further examine the role of Cys392 and Cys405 in facilitating the actions of quinones against human topoisomerase II α , the abilities of the wild-type enzyme, top2 α C170A, top2 α C392A, top2 α C405A, and top2 α C392/405A to religate cleaved DNA in the absence or presence of benzoquinone were determined (Figure 4). In the absence of the quinone, all of the enzymes religated DNA to a similar extent (\sim 50% ligation was observed in 10 s). However, in the presence of benzoquinone, top2 α C392A, top2 α C405A, and top2 α C392/405A exhibited faster rates of religation (\sim 40% ligation in 10 s) compared to either wild-type topoisomerase II α or top2 α C170A (\sim 20% ligation in 10 s). These findings support the conclusions of the preceding section and strongly suggest that adduction of Cys392 and Cys405 by quinones increases levels of DNA cleavage complexes by inhibiting the ability of the enzyme to religate cleaved nucleic acid substrates.

Ability of Benzoquinone To Block the N-Terminal Gate of Mutant Human Topoisomerase II α Enzymes. As discussed above, quinones that are strong topoisomerase II poisons contain multiple electrophilic carbons (i.e., sites of adduction) and block the N-terminal gate of human topoisomerase II α by cross-linking the two protomers of the enzyme (39, 53). This action has no direct effect on the DNA cleavage–religation equilibrium of the enzyme. However, since it traps the non-covalent protein–DNA complex, it increases the proportion of topoisomerase II molecules that contain DNA in their active sites. Therefore, to determine whether any of the cysteine residues identified by mass spectrometry as sites of quinone adduction in topoisomerase II α are involved in blocking the N-terminal gate of the enzyme, DNA binding,

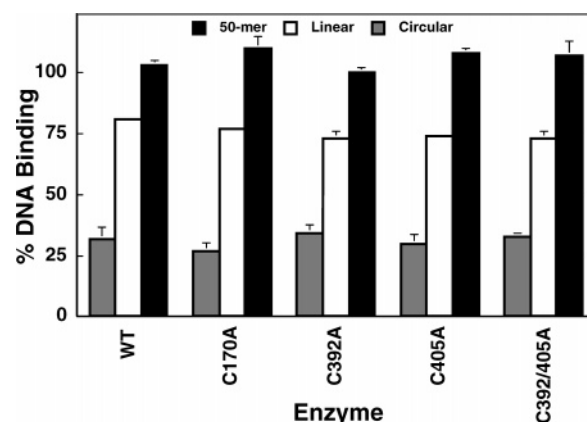


FIGURE 5: Substrate-dependent effects of benzoquinone on mutant topoisomerase II α –DNA binding. Assays employed a negatively supercoiled circular plasmid (gray bars), a linear plasmid (white bars), or a duplex 50-mer oligonucleotide (black bars) as the substrate. Enzymes were incubated with 100 μ M benzoquinone for 5 min prior to the addition of DNA. DNA binding in the absence of benzoquinone was set to 100%. Error bars represent the standard deviation of at least three independent experiments.

protein clamp closing, and protomer cross-linking experiments were performed.

Initial studies examined the ability of quinone-treated enzymes to bind circular and linear forms of DNA. As seen in Figure 5, treatment of wild-type topoisomerase II α with benzoquinone prior to the addition of DNA differentially affects the ability of the enzyme to bind these DNA substrates. By blocking the N-terminal gate of topoisomerase II α , quinones impair the ability of circular DNA to diffuse into the active site in the central annulus of the protein. This action decreased levels of circular DNA binding to \sim 30% of the levels observed in the absence of quinone. In contrast, linear forms of DNA, especially short oligonucleotides (double-stranded 50-mer), are able to enter the “closed clamp” form of the enzyme and bind to the active site (\sim 80 and 100% binding, respectively, as compared to parallel “no quinone” controls). DNA binding results for quinone-treated top2 α C170A, top2 α C392A, top2 α C405A, and top2 α C392/405A were nearly identical to those observed for the wild-type enzyme (Figure 5).

The second set of experiments utilized a “clamp closing” assay. In these experiments, the enzyme was incubated with circular DNA substrates prior to the addition of benzoquinone. Blocking the N-terminal gate traps the circular substrate in the central annulus of topoisomerase II α , generating a noncovalent protein–DNA complex that is stable in 1 M NaCl (67). Benzoquinone treatment increased the concentration of salt-stable protein–DNA complexes \sim 4-fold (from \sim 4.5 to \sim 17% of the total DNA) when wild-type topoisomerase II α was employed (Figure 6). Once again, similar results were observed for all of mutant enzymes. Levels of salt-stable complexes formed with top2 α C392A, top2 α C405A, and top2 α C392/405A were slightly lower (\sim 13–14% of the total DNA) than that seen with the wild-type enzyme in the presence of benzoquinone. However, levels of salt-stable protein complexes formed with these enzymes also were lower (\sim 3.2–3.6% of the total DNA) in the absence of quinone (Figure 6).

The third set of experiments examined the ability of benzoquinone to cross-link the two protomer subunits of

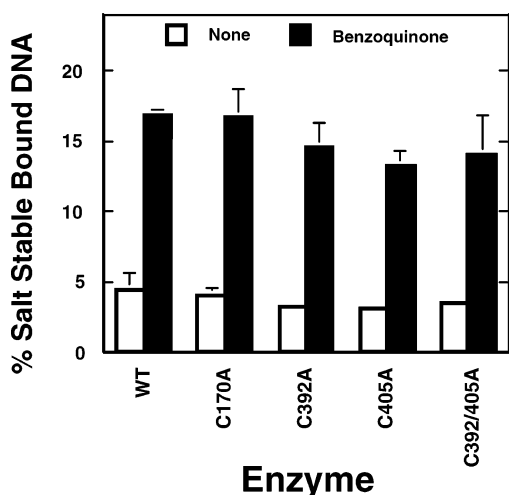


FIGURE 6: Benzoquinone blocks the N-terminal gate of mutant human topoisomerase II α enzymes. Filter binding assays were used to analyze the salt-stable closed clamp of topoisomerase II. Enzyme–DNA complexes were established and further incubated in the absence (left bars) or presence (right bars) of 100 μ M benzoquinone. Samples were applied to glass fiber filters; DNA was eluted by sequential washes in low salt, high salt, and SDS, and eluted samples were subjected to electrophoresis in an agarose gel. Salt-stable noncovalent enzyme–DNA complexes were quantified by the amount of plasmid that did not elute until the SDS wash relative to the total plasmid eluted in all three washes. Error bars represent the standard deviation of at least three independent experiments.

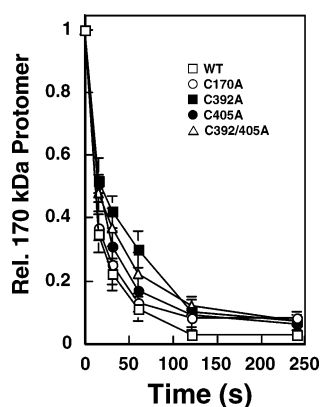


FIGURE 7: Benzoquinone cross-links the protomer subunits of mutant human topoisomerase II α enzymes. Assay mixtures contained 135 nM wild-type enzyme (\square), top2 α C170A (\circ), top2 α C392A (\blacksquare), top2 α C405A (\bullet), or top2 α C392/405A (\triangle) that was treated with 100 μ M benzoquinone for 0–4 min. Samples were subjected to electrophoresis in a polyacrylamide gel, and protein was visualized by Coomassie staining. The level of 170 kDa enzyme protomer that was present in the absence of benzoquinone was set to 1. Error bars represent the standard deviation of at least three independent experiments.

human topoisomerase II α . Exposure of the enzyme to quinones efficiently cross-links the two protomer subunits of the protein homodimer (39). This event is monitored by gel electrophoresis under denaturing conditions and is characterized by the loss of the \sim 170 kDa protomer and the subsequent generation of a new protein species with an apparent molecular mass of \sim 330 kDa. As seen in Figure 7, treatment of wild-type topoisomerase II α as well as the mutant enzymes with benzoquinone resulted in a rapid loss of protein monomer. The half-life for protomer cross-linking (\sim 15 s) was similar for all of the enzymes that were

examined. To address the possibility that all three cysteine residues (Cys170, Cys392, and Cys405) are involved in cross-linking with only one being required at any given time, a triple mutant of topoisomerase II α (top2 α C170/392/405A) was generated. The half-life for protomer cross-linking of this enzyme was similar to that of double mutant top2 α C392/405A (data not shown).

Taken together, the experiments described above provide strong evidence that Cys170, Cys392, and Cys405 are not required for quinones to block the N-terminus of topoisomerase II α or cross-link the two protomer subunits of the protein. Thus, the adduction of Cys392 and Cys405 by benzoquinone appears to enhance DNA scission mediated by topoisomerase II α primarily by inhibiting the ability of the enzyme to religate cleaved nucleic acid molecules.

DISCUSSION

A variety of drug-related and environmental quinone-based metabolites are potent topoisomerase II poisons. These compounds act by adducting the protein, and previous studies suggest that they increase levels of enzyme–DNA cleavage complexes by at least two potentially independent mechanisms (36–39). Quinones act directly on the DNA cleavage–religation equilibrium of topoisomerase II by inhibiting rates of religation. Alternatively, they block the N-terminal gate of the protein by cross-linking its two protomer subunits and thereby increase the levels of cleavage complexes by increasing the local concentration of DNA in the active site of the enzyme. It is not known whether these two effects result from adduction of quinone to the same amino acid residue(s) in topoisomerase II α or whether they are mediated by modification of separate residues.

Although anticancer drugs and other classic topoisomerase II poisons function at the active site of the enzyme (4, 15, 17–19, 71), sites at which quinone-based poisons adduct the protein are not known. Therefore, the present study identified amino acid residues that are modified by quinones and determined their role in the actions of these compounds as topoisomerase II poisons. Four cysteine residues were identified by mass spectrometry as sites of quinone adduction: Cys170, Cys392, Cys405, and Cys455. Mutations (Cys \rightarrow Ala) were individually generated at each of these positions. Only mutations at Cys392 or Cys405 resulted in enzymes with reduced sensitivity to benzoquinone or 4'-Cl-2,5pQ. Top2 α C392A, top2 α C405A, and double mutant top2 α C392/405A all displayed \sim 50% resistance to these compounds. Furthermore, top2 α C392/405A (the only mutant enzyme tested) was \sim 2–4-fold resistant to plumbagin (data not shown). Results of DNA religation and binding studies, together with protein clamp closing and cross-linking experiments, strongly suggest that adduction of Cys392 and Cys405 increases levels of topoisomerase II α -mediated DNA cleavage primarily by inhibiting the ability of the enzyme to reseal nucleic acid breaks.

Studies with top2 α C392A, top2 α C405A, and double mutant top2 α C392/405A indicate that Cys392 and Cys405 both are required for inhibition of DNA religation by quinones. Given the proximity of these two residues (Figure 2), an intriguing possibility is that quinones such as benzoquinone act by forming an intraprotomer cross-link between Cys392 and Cys405. This could help explain why monoad-

ducting quinones, such as plumbagin, are weak topoisomerase II poisons. Future structural studies will be required to explore this issue.

None of the cysteine residues identified in this study appear to be involved in quinone blocking of the N-terminal gate or protomer cross-linking. This finding implies that the effect described above and the inhibition of DNA religation by quinones are mediated by adduction to separate amino acid residues. As monitored by mass spectrometry, 11 of the 13 cysteine residues in human topoisomerase II α were covered by the tryptic digests that were employed. Of the two that were not covered, Cys300 and Cys862, only the former is in the N-terminal domain, and mutagenesis studies indicated that elimination of Cys300 had no effect on quinone sensitivity. It is notable that the N-terminal peptide of topoisomerase II α was not covered in our mass spectrometry study. In light of the fact that N-terminal amines often exhibit high reactivity, it is possible that this residue is involved in cross-linking the protomer subunits of the enzyme. High-resolution mass spectrometry studies currently are underway to address this possibility.

In conclusion, quinones are topoisomerase II poisons that act by adducting the enzyme. Results of this study indicate that adduction of Cys392 and Cys405 is important for the actions of quinones and increases levels of enzyme–DNA cleavage complexes primarily by inhibiting DNA religation.

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SUPPORTING INFORMATION AVAILABLE

Representative spectra of plumbagin-adducted peptides of human topoisomerase II α . This material is available free of charge via the Internet at <http://pubs.acs.org>.

REFERENCES

- Wang, J. C. (1996) DNA Topoisomerases, *Annu. Rev. Biochem.* 65, 635–692.
- Nitiss, J. L. (1998) Investigating the biological functions of DNA topoisomerases in eukaryotic cells, *Biochim. Biophys. Acta* 1400, 63–81.
- Wang, J. C. (1998) Moving one DNA double helix through another by a type II DNA topoisomerase: The story of a simple molecular machine, *Q. Rev. Biophys.* 31, 107–144.
- Fortune, J. M., and Osheroff, N. (2000) Topoisomerase II as a target for anticancer drugs: When enzymes stop being nice, *Prog. Nucleic Acid Res. Mol. Biol.* 64, 221–253.
- Champoux, J. J. (2001) DNA topoisomerases: Structure, function, and mechanism, *Annu. Rev. Biochem.* 70, 369–413.
- Wang, J. C. (2002) Cellular roles of DNA topoisomerases: A molecular perspective, *Nat. Rev. Mol. Cell Biol.* 3, 430–440.
- Velez-Cruz, R., and Osheroff, N. (2004) DNA Topoisomerases: Type II, in *Encyclopedia of Molecular Biology* (Lennarz, W., and Lane, M. D., Eds.) pp 806–811, Elsevier Science, San Diego.
- Sander, M., and Hsieh, T. (1983) Double strand DNA cleavage by type II DNA topoisomerase from *Drosophila melanogaster*, *J. Biol. Chem.* 258, 8421–8428.
- Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M., and Chen, G. L. (1983) Cleavage of DNA by mammalian DNA topoisomerase II, *J. Biol. Chem.* 258, 15365–15370.
- Zechiedrich, E. L., Christiansen, K., Andersen, A. H., Westergaard, O., and Osheroff, N. (1989) Double-stranded DNA cleavage/religation reaction of eukaryotic topoisomerase II: Evidence for a nicked DNA intermediate, *Biochemistry* 28, 6229–6236.
- Kaufmann, S. H. (1998) Cell death induced by topoisomerase-targeted drugs: More questions than answers, *Biochim. Biophys. Acta* 1400, 195–211.
- Kaufmann, S. H., Gore, S. D., Miller, C. B., Jones, R. J., Zwelling, L. A., Schneider, E., Burke, P. J., and Karp, J. E. (1998) Topoisomerase II and the response to antileukemic therapy, *Leuk. Lymphoma* 29, 217–237.
- Rowley, J. D. (1998) The critical role of chromosome translocations in human leukemias, *Annu. Rev. Genet.* 32, 495–519.
- Felix, C. A. (1998) Secondary leukemias induced by topoisomerase-targeted drugs, *Biochim. Biophys. Acta* 1400, 233–255.
- Wilstermann, A. M., and Osheroff, N. (2003) Stabilization of eukaryotic topoisomerase II–DNA cleavage complexes, *Curr. Top. Med. Chem.* 3, 321–338.
- Sordet, O., Khan, Q. A., Kohn, K. W., and Pommier, Y. (2003) Apoptosis induced by topoisomerase inhibitors, *Curr. Med. Chem. Anti-Cancer Agents* 3, 271–290.
- Li, T. K., and Liu, L. F. (2001) Tumor cell death induced by topoisomerase-targeting drugs, *Annu. Rev. Pharmacol. Toxicol.* 41, 53–77.
- Walker, J. V., and Nitiss, J. L. (2002) DNA topoisomerase II as a target for cancer chemotherapy, *Cancer Invest.* 20, 570–589.
- Baldwin, E. L., and Osheroff, N. (2005) Etoposide, topoisomerase II and cancer, *Curr. Med. Chem. Anti-Cancer Agents* 5, 363–372.
- Adlercreutz, H., Markkanen, H., and Watanabe, S. (1993) Plasma concentrations of phyto-oestrogens in Japanese men, *Lancet* 342, 1209–1210.
- Lamartiniere, C. A. (2000) Protection against breast cancer with genistein: A component of soy, *Am. J. Clin. Nutr.* 71, 1705S–1709S.
- Sarkar, F. H., Adsule, S., Padhye, S., Kulkarni, S., and Li, Y. (2006) The role of genistein and synthetic derivatives of isoflavone in cancer prevention and therapy, *Mini-Rev. Med. Chem.* 6, 401–407.
- Siddiqui, I. A., Adhami, V. M., Saleem, M., and Mukhtar, H. (2006) Beneficial effects of tea and its polyphenols against prostate cancer, *Mol. Nutr. Food Res.* 50, 130–143.
- DeVore, R., Whitlock, J., Hainsworth, T., and Johnson, D. (1989) Therapy-related acute nonlymphocytic leukemia with monocytic features and rearrangement of chromosome 11q, *Ann. Int. Med.* 110, 740–742.
- Ratain, M. J., and Rowley, J. D. (1992) Therapy-related acute myeloid leukemia secondary to inhibitors of topoisomerase II: From the bedside to the target genes, *Ann. Oncol.* 3, 107–111.
- Smith, M. A., Rubinstein, L., Anderson, J. R., Arthur, D., Catalano, P. J., Freidlin, B., Heyn, R., Khayat, A., Krailo, M., Land, V. J., Miser, J., Shuster, J., and Vena, D. (1999) Secondary leukemia or myelodysplastic syndrome after treatment with epipodophyllotoxins, *J. Clin. Oncol.* 17, 569–577.
- Pui, C. H., and Relling, M. V. (2000) Topoisomerase II inhibitor-related acute myeloid leukaemia, *Br. J. Haematol.* 109, 13–23.
- Felix, C. A. (2001) Leukemias related to treatment with DNA topoisomerase II inhibitors, *Med. Pediatr. Oncol.* 36, 525–535.
- Leone, G., Voso, M. T., Sica, S., Morosetti, R., and Pagano, L. (2001) Therapy related leukemias: Susceptibility, prevention and treatment, *Leuk. Lymphoma* 41, 255–276.
- Ross, J. A., Potter, J. D., and Robison, L. L. (1994) Infant leukemia, topoisomerase II inhibitors, and the MLL gene, *J. Natl. Cancer Inst.* 86, 1678–1680.
- Ross, J. A., Potter, J. D., Reaman, G. H., Pendergrass, T. W., and Robison, L. L. (1996) Maternal exposure to potential inhibitors of DNA topoisomerase II and infant leukemia (United States): A report from the Children's Cancer Group, *Cancer, Causes Control* 7, 581–590.
- Ross, J. A. (2000) Dietary flavonoids and the MLL gene: A pathway to infant leukemia? *Proc. Natl. Acad. Sci. U.S.A.* 97, 4411–4413.
- Strick, R., Strissel, P. L., Borgers, S., Smith, S. L., and Rowley, J. D. (2000) Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia, *Proc. Natl. Acad. Sci. U.S.A.* 97, 4790–4795.
- Spector, L. G., Xie, Y., Robison, L. L., Heerema, N. A., Hilden, J. M., Lange, B., Felix, C. A., Davies, S. M., Slavin, J., Potter, J. D., Blair, C. K., Reaman, G. H., and Ross, J. A. (2005) Maternal diet and infant leukemia: The DNA topoisomerase II inhibitor hypothesis: A report from the children's oncology group, *Cancer Epidemiol., Biomarkers Prev.* 14, 651–655.

35. Wang, H., Mao, Y., Chen, A. Y., Zhou, N., LaVoie, E. J., and Liu, L. F. (2001) Stimulation of topoisomerase II-mediated DNA damage via a mechanism involving protein thiolation, *Biochemistry* 40, 3316–3323.
36. Bender, R. P., Lindsey, R. H., Jr., Burden, D. A., and Osheroff, N. (2004) N-Acetyl-p-benzoquinone imine, the toxic metabolite of acetaminophen, is a topoisomerase II poison, *Biochemistry* 43, 3731–3739.
37. Lindsey, R. H., Jr., Bromberg, K. D., Felix, C. A., and Osheroff, N. (2004) 1,4-Benzoquinone is a topoisomerase II poison, *Biochemistry* 43, 7563–7574.
38. Lindsey, R. H., Jr., Bender, R. P., and Osheroff, N. (2005) Effects of benzene metabolites on DNA cleavage mediated by human topoisomerase II α : 1,4-Hydroquinone is a topoisomerase II poison, *Chem. Res. Toxicol.* 18, 761–770.
39. Bender, R. P., Lehmler, H. J., Robertson, L. W., Ludewig, G., and Osheroff, N. (2006) Polychlorinated Biphenyl Quinone Metabolites Poison Human Topoisomerase II α : Altering Enzyme Function by Blocking the N-Terminal Protein Gate, *Biochemistry* 45, 10140–10152.
40. Ludewig, G., Dogra, S., and Glatt, H. (1989) Genotoxicity of 1,4-benzoquinone and 1,4-naphthoquinone in relation to effects on glutathione and NAD(P)H levels in V79 cells, *Environ. Health Perspect.* 82, 223–228.
41. Smith, M. T. (1996) The mechanism of benzene-induced leukemia: A hypothesis and speculations on the causes of leukemia, *Environ. Health Perspect.* 104 (Suppl. 6), 1219–1225.
42. Sze, C. C., Shi, C. Y., and Ong, C. N. (1996) Cytotoxicity and DNA strand breaks induced by benzene and its metabolites in Chinese hamster ovary cells, *J. Appl. Toxicol.* 16, 259–264.
43. Nakayama, A., Koyoshi, S., Morisawa, S., and Yagi, T. (2000) Comparison of the mutations induced by p-benzoquinone, a benzene metabolite, in human and mouse cells, *Mutat. Res.* 470, 147–153.
44. Ludewig, G. (2001) Cancer Initiation by PCBs, in *PCBs, Recent Advances in Environmental Toxicology and Health Effects* (Robertson, L. W., and Hansen, L. G., Eds.) pp 337–354, University Press of Kentucky, Lexington, KY.
45. Wiemels, J. L., Pagnamenta, A., Taylor, G. M., Eden, O. B., Alexander, F. E., and Greaves, M. F. (1999) A lack of a functional NAD(P)H:quinone oxidoreductase allele is selectively associated with pediatric leukemias that have MLL fusions. United Kingdom Childhood Cancer Study Investigators, *Cancer Res.* 59, 4095–4099.
46. Larson, R. A., Wang, Y., Banerjee, M., Wiemels, J., Hartford, C., Le Beau, M. M., and Smith, M. T. (1999) Prevalence of the inactivating 609C→T polymorphism in the NAD(P)H:quinone oxidoreductase (NQO1) gene in patients with primary and therapy-related myeloid leukemia, *Blood* 94, 803–807.
47. Smith, M. T. (1999) Benzene, NQO1, and genetic susceptibility to cancer, *Proc. Natl. Acad. Sci. U.S.A.* 96, 7624–7626.
48. Smith, M. T., Wang, Y., Kane, E., Rollinson, S., Wiemels, J. L., Roman, E., Roddam, P., Cartwright, R., and Morgan, G. (2001) Low NAD(P)H:quinone oxidoreductase 1 activity is associated with increased risk of acute leukemia in adults, *Blood* 97, 1422–1426.
49. Smith, M. T., Wang, Y., Skibola, C. F., Slater, D. J., Lo Nigro, L., Nowell, P. C., Lange, B. J., and Felix, C. A. (2002) Low NAD(P)H:quinone oxidoreductase activity is associated with increased risk of leukemia with MLL translocations in infants and children, *Blood* 100, 4590–4593.
50. Chen, H., and Eastmond, D. A. (1995) Topoisomerase inhibition by phenolic metabolites: A potential mechanism for benzene's clastogenic effects, *Carcinogenesis* 16, 2301–2307.
51. Hutt, A. M., and Kalf, G. F. (1996) Inhibition of human DNA topoisomerase II by hydroquinone and p-benzoquinone, reactive metabolites of benzene, *Environ. Health Perspect.* 104 (Suppl. 6), 1265–1269.
52. Frantz, C. E., Chen, H., and Eastmond, D. A. (1996) Inhibition of human topoisomerase II in vitro by bioactive benzene metabolites, *Environ. Health Perspect.* 104 (Suppl. 6), 1319–1323.
53. Srinivasan, A., Robertson, L. W., and Ludewig, G. (2002) Sulfhydryl binding and topoisomerase inhibition by PCB metabolites, *Chem. Res. Toxicol.* 15, 497–505.
54. Eastmond, D. A., Mondrala, S. T., and Hasegawa, L. (2005) Topoisomerase II inhibition by myeloperoxidase-activated hydroquinone: A potential mechanism underlying the genotoxic and carcinogenic effects of benzene, *Chem.-Biol. Interact.* 153–154, 207–216.
55. Worland, S. T., and Wang, J. C. (1989) Inducible overexpression, purification, and active site mapping of DNA topoisomerase II from the yeast *Saccharomyces cerevisiae*, *J. Biol. Chem.* 264, 4412–4416.
56. Elsea, S. H., Hsiung, Y., Nitiss, J. L., and Osheroff, N. (1995) A yeast type II topoisomerase selected for resistance to quinolones. Mutation of histidine 1012 to tyrosine confers resistance to nonintercalative drugs but hypersensitivity to ellipticine, *J. Biol. Chem.* 270, 1913–1920.
57. Kingma, P. S., Greider, C. A., and Osheroff, N. (1997) Spontaneous DNA lesions poison human topoisomerase II α and stimulate cleavage proximal to leukemic 11q23 chromosomal breakpoints, *Biochemistry* 36, 5934–5939.
58. Amaro, A. R., Oakley, G. G., Bauer, U., Spielmann, H. P., and Robertson, L. W. (1996) Metabolic activation of PCBs to quinones: Reactivity toward nitrogen and sulfur nucleophiles and influence of superoxide dismutase, *Chem. Res. Toxicol.* 9, 623–629.
59. Eng, J. K., McCormack, A. L., and Yates, J. R. I. (1994) An approach to correlate tandem mass-spectral data of peptides with amino-acid-sequences in a protein database, *J. Am. Soc. Mass Spectrom.* 5, 976–998.
60. Hansen, B. T., Davey, S. W., Ham, A. J., and Liebler, D. C. (2005) P-Mod: An algorithm and software to map modifications to peptide sequences using tandem MS data, *J. Proteome Res.* 4, 358–368.
61. Wasserman, R. A., Austin, C. A., Fisher, L. M., and Wang, J. C. (1993) Use of yeast in the study of anticancer drugs targeting DNA topoisomerases: Expression of a functional recombinant human DNA topoisomerase II α in yeast, *Cancer Res.* 53, 3591–3596.
62. O'Reilly, E. K., and Kreuzer, K. N. (2002) A unique type II topoisomerase mutant that is hypersensitive to a broad range of cleavage-inducing antitumor agents, *Biochemistry* 41, 7989–7997.
63. Fortune, J. M., and Osheroff, N. (1998) Merbarone inhibits the catalytic activity of human topoisomerase II α by blocking DNA cleavage, *J. Biol. Chem.* 273, 17643–17650.
64. Byl, J. A., Fortune, J. M., Burden, D. A., Nitiss, J. L., Utsugi, T., Yamada, Y., and Osheroff, N. (1999) DNA topoisomerases as targets for the anticancer drug TAS-103: Primary cellular target and DNA cleavage enhancement, *Biochemistry* 38, 15573–15579.
65. Fortune, J. M., Dickey, J. S., Lavrukhin, O. V., Van Etten, J. L., Lloyd, R. S., and Osheroff, N. (2002) Site-specific DNA cleavage by *Chlorella* virus topoisomerase II, *Biochemistry* 41, 11761–11769.
66. Fortune, J. M., Lavrukhin, O. V., Gurnon, J. R., Van Etten, J. L., Lloyd, R. S., and Osheroff, N. (2001) Topoisomerase II from *Chlorella* virus PBCV-1 has an exceptionally high DNA cleavage activity, *J. Biol. Chem.* 276, 24401–24408.
67. Vaughn, J., Huang, S., Wessel, I., Sorensen, T. K., Hsieh, T., Jensen, L. H., Jensen, P. B., Sehested, M., and Nitiss, J. L. (2005) Stability of the topoisomerase II closed clamp conformation may influence DNA-stimulated ATP hydrolysis, *J. Biol. Chem.* 280, 11920–11929.
68. Berger, J. M., Gamblin, S. J., Harrison, S. C., and Wang, J. C. (1996) Structure and mechanism of DNA topoisomerase II, *Nature* 379, 225–232.
69. Classen, S., Olland, S., and Berger, J. M. (2003) Structure of the topoisomerase II ATPase region and its mechanism of inhibition by the chemotherapeutic agent ICRF-187, *Proc. Natl. Acad. Sci. U.S.A.* 100, 10629–10634.
70. Hasinoff, B. B., Wu, X., Krokhin, O. V., Ens, W., Standing, K. G., Nitiss, J. L., Sivaram, T., Giorgianni, A., Yang, S., Jiang, Y., and Yalowich, J. C. (2005) Biochemical and proteomics approaches to characterize topoisomerase II α cysteines and DNA as targets responsible for cisplatin-induced inhibition of topoisomerase II α , *Mol. Pharmacol.* 67, 937–947.
71. Pommier, Y., and Marchand, C. (2005) Interfacial inhibitors of protein-nucleic acid interactions, *Curr. Med. Chem. Anti-Cancer Agents* 5, 421–429.