Exocyclic DNA Lesions Stimulate DNA Cleavage Mediated by Human Topoisomerase IIα in Vitro and in Cultured Cells†

Renier Vélez-Cruz,‡ James N. Riggins,‡ J. Scott Daniels,‡ Hongliang Cai,‡,§ F. Peter Guengerich,‡,§,|| Lawrence J. Marnett,‡,§,|| and Neil Osheroff*,‡,||,⊥

Departments of Biochemistry and Medicine (Hematology/Oncology), Center in Molecular Toxicology, and Vanderbilt Institute of Chemical Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

Received October 8, 2004; Revised Manuscript Received December 31, 2004

ABSTRACT: DNA adducts are mutagenic and clastogenic. Because of their harmful nature, lesions are recognized by many proteins involved in DNA repair. However, mounting evidence suggests that lesions also are recognized by proteins with no obvious role in repair processes. One such protein is topoisomerase II, an essential enzyme that removes knots and tangles from the DNA. Because topoisomerase II generates a protein-linked double-stranded DNA break during its catalytic cycle, it has the potential to fragment the genome. Previous studies indicate that abasic sites and other lesions that distort the double helix stimulate topoisomerase II-mediated DNA cleavage. Therefore, to further explore interactions between DNA lesions and the enzyme, the effects of exocyclic adducts on DNA cleavage mediated by human topoisomerase IIα were determined. When located within the four-base overhang of a topoisomerase II cleavage site (at the +2 or +3 position 3′ relative to the scissile bond), 3,N^4-ethenodeoxycytidine, 3,N^4-etheno-2′-ribocytidine, 1,N^2-ethenodeoxyguanosine, pyrimido[1,2-*a*]purin-10(3*H*)-one deoxyribose (M_1dG), and 1,N^2-propanodeoxyguanosine increased DNA scission ∼5−17-fold. Enhanced cleavage did not result from an increased affinity of topoisomerase IIα for adducted DNA or a decreased rate of religation. Therefore, it is concluded that these exocyclic lesions act by accelerating the forward rate of enzyme-mediated DNA scission. Finally, treatment of cultured human cells with 2-chloroacetaldehyde, a reactive metabolite of vinyl chloride that generates etheno adducts, increased cellular levels of DNA cleavage by topoisomerase IIα. This finding suggests that type II topoisomerases interact with exocyclic DNA lesions in physiological systems.

Despite the multiple defense mechanisms that mammalian cells use to protect their genetic material, the human genome is under constant attack by a plethora of endogenous and environmental chemicals that damage DNA. Alkylating agents are among the most common of these DNA-reactive chemicals and can be either monofunctional or bifunctional in nature (*1*, *2*). The former generate singly modified bases (i.e., methylated, ethylated), while the latter often produce exocyclic DNA adducts. Alkylated bases can be highly mutagenic and carcinogenic (*3*).The interaction of repair proteins, DNA polymerases, and RNA polymerases with adducted bases has been documented (*4*−*8*). However, relatively little is known about how damaged bases affect the actions of other nuclear proteins. In this regard, DNA topoisomerases are among the few enzymes that have been investigated. When abasic sites or other specific lesions are located in proximity to a topoisomerase I (*9*−*14*) or topoisomerase II (*15*−*21*) cleavage site, they often have a dramatic effect on DNA scission mediated by these two enzymes.

DNA topoisomerases are enzymes that control the topological state of the double helix (*22*). Topoisomerase I regulates levels of DNA under- and overwinding by generating transient enzyme-linked single-stranded breaks in the genetic material followed by controlled unwinding of the double helix (*23*). This enzyme plays an important role in maintaining the global superhelical density of DNA and is intimately associated with processes such as DNA replication and transcription (*23*−*25*). Topoisomerase II modulates levels of DNA under- and overwinding and removes knots and tangles from the genetic material by passing an intact double helix through a transient double-stranded break that it generates in a separate segment of DNA (*26*−*29*). This enzyme is required for chromosomal segregation and the resolution of mitotic recombination products (*24*, *25*, *28*, *30*).

To maintain genomic integrity while topoisomerases generate transient DNA breaks, these enzymes form covalent bridges between active site tyrosyl residues and the termini of the cleaved DNA molecules (*26*−*30*). These covalent enzyme−DNA intermediates, known as *cleavage complexes*, normally are present at very low levels and are tolerated by the cell. If the physiological concentrations of cleavage

† This work was supported by National Institutes of Health Grants GM53960 (N.O.), CA87819 and ES00267 (L.J.M.), CA44353, ES10375, and ES00267 (F.P.G.), and ES05919 (H.C.). R.V.-C. and J.N.R. were trainees under National Institutes of Health Grants CA09385 and ES07028, respectively.

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

‡ Department of Biochemistry.

§ Center in Molecular Toxicology.

|| Vanderbilt Institute of Chemical Biology.

⊥ Department of Medicine (Hematology/Oncology).

10.1021/bi0478289 CCC: $30.25
Published on Web 02/12/2005

complexes rise above normal levels, they become roadblocks for DNA tracking enzymes such as polymerases and helicases. Collisions between DNA tracking enzymes and cleavage complexes convert transient topoisomerase-mediated cleavage events to permanent DNA breaks that are toxic to cells (*26*, *28*−*30*). Agents that increase the levels of topoisomerase−DNA cleavage complexes are referred to as topoisomerase *poisons*, because they transform these important enzymes into potent cellular toxins that fragment the genome (*28*−*33*).

Some topoisomerase I poisons, such as topotecan, and some topoisomerase II poisons, such as etoposide, are in wide clinical use as anticancer agents (*34*, *35*). These drugs are front-line therapies for a variety of human malignancies. In contrast, exposure to environmental topoisomerase II poisons such as benzoquinone (a benzene metabolite) or certain flavanoids has been linked to the initiation of specific types of leukemia (*36*−*38*). Leukemias associated with topoisomerase II poisons generally display chromosomal translocations with breakpoints that originate within the *MLL* gene at chromosomal band 11q23 (*36*−*42*).

Previous studies demonstrated that abasic sites and DNA adducts are often potent poisons of topoisomerase I (*9*−*14*) or II (*15*−*21*) in vitro. Lesions act in a position-specific manner. The scissile bonds cleaved by topoisomerase II on the two strands of the double helix are staggered by four bases. When abasic sites are located within this four-base stagger, they stimulate topoisomerase II-mediated DNA cleavage as much as 20-fold (*18*). In contrast, when they are located immediately outside of the scissile bonds, they often inhibit DNA cleavage (*18*).

In addition, there appears to be a correlation between the ability of a DNA adduct to distort the double helix and its potential to act as a topoisomerase II poison (*21*). Abasic sites and ϵdA,[1] both of which induce kinks in DNA (*21*), increase enzyme-mediated DNA cleavage ∼10−20-fold. In contrast, adducts such as 8-oxodeoxyguanosine, O^6-methyldeoxyguanosine, 8-oxodeoxyadenosine, and N^6-methyldeoxyadenosine, which induce little distortion in DNA, have relatively small (if any) effects on DNA cleavage (*21*).

Although some DNA damaging agents induce topoisomerase I-mediated DNA cleavage in cultured cells (*13*, *43*), DNA lesions have not as yet been shown to act as topoisomerase II poisons in human cells. Therefore, the present study investigated the effects of alkylated bases on DNA scission mediated by human topoisomerase IIα. Results indicate that a variety of exocyclic base adducts, including ϵdC, ϵdG, M_1dG, and PdG (see Figure 1), enhance DNA cleavage by the type II enzyme ∼5−17-fold. In addition, 2-chloroacetaldehyde (a reactive product of vinyl chloride metabolism), which induces etheno base adducts in DNA (*44*, *45*), is a potent topoisomerase IIα poison in cultured human cells.

EXPERIMENTAL PROCEDURES

Enzymes and Materials. Human topoisomerase IIα was expressed in *Saccharomyces cerevisiae* and purified as described previously (*17*). Etoposide was obtained from Sigma (St. Louis, MO) and was prepared as a 20 mM stock in 100% DMSO and stored at 4 °C. The alkylating agent 2-chloroacetaldehyde [50% (v/v) aqueous solution] also was obtained from Sigma and was stored at room temperature. All other chemicals were of analytical reagent grade.

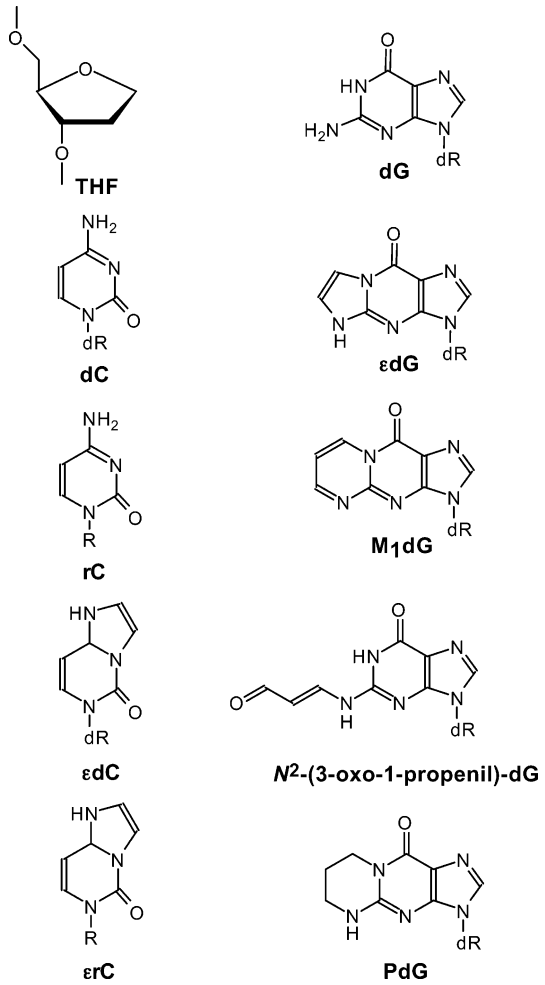

FIGURE 1: Structures of DNA lesions. The structures of the DNA lesions used in this study are shown. THF is used as an analogue of an abasic site. Etheno adducts are produced endogenously by lipid peroxidation byproducts (*51*, *89*) and exogenously by exposure to vinyl chloride (*52*). M_1dG is produced endogenously by malondialdehyde and base propenals (ring-closed and ring-opened structures) (*57*, *58*), and PdG is an unnatural adduct used commonly as a M_1dG analogue (*61*−*63*).

Site-Specific DNA Cleavage Induced by DNA Lesions. DNA sites cleaved by human topoisomerase IIα in oligonucleotide substrates were determined as described previously (*18*). A 42-mer oligonucleotide corresponding to residues 1039−1081 of the *MLL* gene and its complementary strand were prepared on an Applied Biosystems DNA synthesizer. This substrate spans a previously mapped leukemic breakpoint at position 1067. The sequences of the top and bottom strands were 5′-ATGATTGTACCACTG-CAG↓TCCAGCCTGGGTGACAAAGC-AAAA-3′ and 5′-TTTTGCTTTGTCACCCAGGC↓TGGACTGCAGTGGTAC-AATCAT-3′, respectively. This substrate contains a single cleavage site for topoisomerase II that has been well characterized (*17*, *39*). The nomenclature of the positions on the top and bottom strands is assigned as follows: positions 5′ to the cleavage site are named with negative

[1] Abbreviations: ϵdC, 3,N^4-ethenodeoxycytidine; ϵrC, 3,N^4-etheno-2′-ribocytidine; ϵdG, 1,N^2-ethenodeoxyguanosine; M_1dG, pyrimido[1,2-*a*]purin-10(3*H*)-one deoxyribose; PdG, 1,N^2-propanodeoxyguanosine; ϵdA, 1,N^6-ethenodeoxyadenosine; rC, 2′-ribocytidine; THF, tetrahydrofuran; BER, base excision repair; APE1, AP endonuclease 1.

numbers, whereas positions 3′ to the cleavage site are named with positive numbers. Scissile bonds are located between the −1 and +1 bases and are denoted by arrows.

Adducted DNA bases were inserted in the oligonucleotide at specific positions using phosphoramidite chemistry for ϵdC (Chem Genes), ϵrC, rC, and THF (Glen Research). The M_1dG, PdG, and ϵdG lesioned oligonucleotides were synthesized as previously described (*46*−*49*). Unmodified oligonucleotides were labeled on the 5′-termini using T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]-ATP (ICN) and gel-purified as described previously (*18*). In all cases, double-stranded DNA substrates were generated by annealing equimolar amounts of complementary oligonucleotides at 70 °C for 10 min and cooling to 25 °C.

Reaction mixtures contained 220 nM human topoisomerase IIα and 100 nM double-stranded oligonucleotide in 20 μL of cleavage buffer [10 mM Tris-HCl (pH 7.9), 100 mM KCl, 5 mM $MgCl_2$, 0.1 mM NaEDTA, and 2.5% glycerol (v/v)]. Reactions were started by the addition of the enzyme, and mixtures were incubated at 37 °C for 10 min. DNA cleavage products were trapped by the addition of 2 μL of 10% SDS followed by 1 μL of 375 mM NaEDTA (pH 8.0). Samples were digested with proteinase K (2 μL of a 0.8 mg/mL solution) for 30 min at 37 °C, precipitated twice in 100% ethanol, rinsed once with 70% ethanol, dried, and resuspended in loading buffer [40% formamide (v/v), 8.4 mM EDTA, 0.02% bromophenol blue (w/v), and 0.02% xylene cyanole FF (w/v)]. DNA cleavage products were resolved by electrophoresis in 7 M urea and 14% polyacrylamide gels in 100 mM Tris−borate (pH 8.3) and 2 mM NaEDTA and were visualized and quantified on a Bio-Rad molecular imager FX. Topoisomerase II-mediated relative DNA cleavage was calculated by dividing the percent DNA scission of the adducted oligonucleotide by the percent DNA scission of the corresponding unmodified oligonucleotide.

DNA cleavage was monitored on the strand opposite the DNA lesion (i.e., the 5′-terminal radioactive label was incorporated on the undamaged DNA strand) in order to maintain a consistent level of labeling from experiment to experiment. Previous studies have demonstrated that a similar effect on DNA cleavage is observed on both strands of the double helix in damage-containing oligonucleotide substrates (*16*, *50*).

DNA Religation Mediated by Topoisomerase IIα. DNA religation assays were carried out by a modification of the procedure of Kingma et al. (*18*). DNA cleavage/religation equilibria were established in cleavage buffer as described in the preceding section with the exception that 5 mM $MgCl_2$ in the reaction buffer was replaced by 5 mM $CaCl_2$. Topoisomerase II−DNA cleavage complexes were trapped by the addition of NaEDTA (6 mM final concentration). NaCl was added (500 mM final concentration) to prevent recleavage. Religation was initiated by the addition of $MgCl_2$ (0.1 mM final concentration) and terminated at times up to 60 s by the addition of 2 μL of 10% SDS. Samples were analyzed as described above. The apparent first-order rate of DNA religation was determined by quantifying the loss of the cleaved DNA product.

Topoisomerase IIα−DNA Binding Affinity. The effects of lesions on the affinity of human topoisomerase IIα for DNA were monitored by a competitive nitrocellulose filter-binding assay. Nitrocellulose filters (0.45 μm, Millipore) were equilibrated in binding buffer [10 mM Tris-HCl (pH 7.9), 40 mM KCl, 0.1 mM NaEDTA, and 2.5% glycerol]. Assays were performed in the absence of a divalent cation to avoid topoisomerase II-mediated DNA cleavage. Binding was initiated by the addition of 220 nM human topoisomerase IIα to a mixture that contained 50 nM ^{32}P-labeled unmodified oligonucleotide and 0−200 nM cold competitor oligonucleotide in binding buffer (20 μL total volume). Binding mixtures were incubated at 37 °C for 10 min, transferred to nitrocellulose filters, and washed three times with binding buffer. Filters were placed in 8 mL of Econo-Safe scintillation fluid (Research Product International), and the amount of ^{32}P-labeled unmodified oligonucleotide that remained bound to the filter was quantified using a Beckman LS 5000TD scintillation counter.

DNA Cleavage Mediated by Topoisomerase IIα in Cultured Human Cells. Human CEM, MDA MB 231, and MCF-7 cells were obtained from ATCC. All cell lines were cultured under 5% CO_2 at 37 °C in RPMI 1640 medium (Cellgro by Mediatech, Inc.), containing 10% heat-inactivated fetal bovine serum (Hyclone) and 2 mM glutamine (Cellgro by Mediatech, Inc.). The in vivo complex of enzyme (ICE) bioassay (as modified on the TopoGEN, Inc., website) was used to determine the effects of 2-chloroacetaldehyde on topoisomerase IIα-associated DNA breaks in treated cells. Exponentially growing cultures were treated with 2-chloroacetaldehyde for times up to 4 h or with etoposide for 1 h for comparison. Human CEM leukemia cells ($\sim 5 \times 10^6$) were harvested by centrifugation. Human MCF-7 and MDA MB 231 breast cancer cells ($\sim 5 \times 10^6$) were incubated with trypsin prior to centrifugation. Following centrifugation, cells were lysed by the immediate addition of 3 mL of 1% sarkosyl in TE (pH 7.5). Following gentle douncing, lysates were layered onto a 2 mL cushion of CsCl (1.5 g/mL) and centrifuged at 80000 rpm for 5.5 h at 20 °C. DNA pellets were isolated, resuspended in 5 mM Tris-HCl (pH 8.0) and 0.5 mM EDTA, and blotted onto nitrocellulose membranes using a Schleicher and Schuell slot-blot apparatus. Covalent complexes formed between topoisomerase IIα and DNA were detected using a polyclonal antibody directed against human topoisomerase IIα (Kiamaya Biochemical Co.) at a 1:2000 dilution. The secondary antibody used was anti-rabbit IgG conjugated to horseradish peroxidase at a 1:4000 dilution. The amount of cleavage complexes was determined using an ECL kit (Amersham). Results were quantified using an Alpha Innotech digital imaging system (San Leandro, CA).

RESULTS

Effects of Exocyclic Base Adducts on DNA Cleavage Mediated by Human Topoisomerase IIα. To further explore the potential of DNA lesions to act as topoisomerase II poisons, the effects of a number of exocyclic base adducts (Figure 1) on DNA cleavage mediated by human topoisomerase IIα were determined. The oligonucleotide substrate used for these studies contains a single well-characterized cleavage site for human topoisomerase IIα (*17*). The sequence is derived from the breakpoint cluster region of the *MLL* gene at chromosomal band 11q23 and contains a leukemic chromosomal translocation breakpoint identified in a patient that had been treated with etoposide (*39*). Since

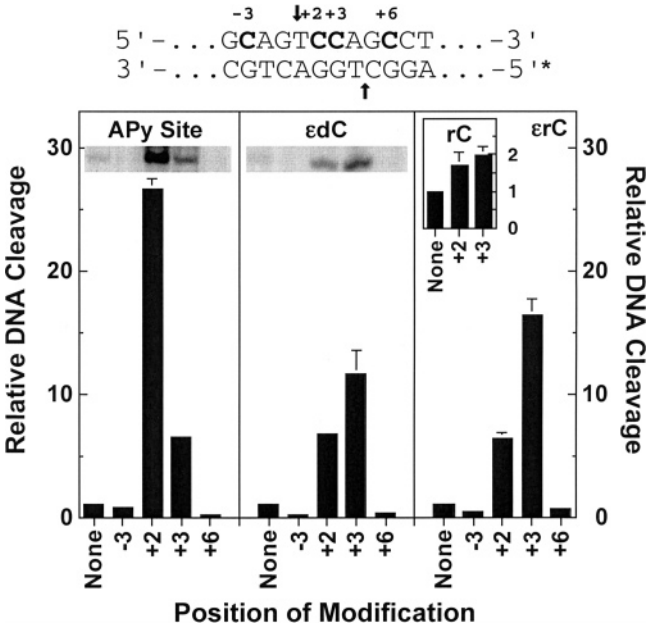

FIGURE 2: ϵdC and ϵrC adducts are position-specific topoisomerase IIα poisons. The central sequence of the 42-mer oligonucleotide substrate used to monitor cleavage of the bottom strand is shown. Boldface indicates the modified positions. The asterisk denotes the position of the 5′-radiolabel, and the arrows indicate the points of topoisomerase II-mediated DNA cleavage. The effects of ϵdC or ϵrC adducts as well as the corresponding apyrimidinic (APy) sites on DNA cleavage mediated by human topoisomerase IIα are shown. The positions of adducts relative to the scissile bonds are given. The scissile bonds are between the −1 and +1 bases. The effects of ribonucleotides (rC) are shown for comparison in the inset. Relative DNA cleavage was calculated by normalizing levels of scission of the unmodified oligonucleotide (None) to 1. Error bars represent the standard deviations of three independent experiments. Representative autoradiograms showing the DNA cleavage product generated in the presence of APy and ϵdC lesions are included above the corresponding bar.

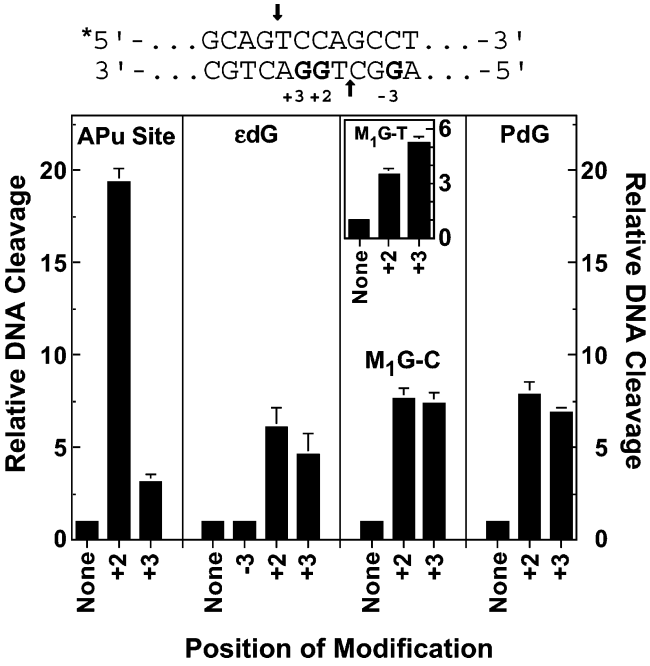

FIGURE 3: ϵdG, M_1dG, and PdG are position-specific poisons of human topoisomerase IIα. The central sequence of the 42-mer oligonucleotide substrate used to monitor cleavage of the top strand is shown as in Figure 2. Boldface indicates the modified positions. The effects of ϵdG, M_1dG, or PdG adducts as well as the corresponding apurinic site (APu) on DNA cleavage mediated by topoisomerase IIα are shown. Results for M_1dG opposite to thymine are shown for comparison in the inset. Relative DNA cleavage was calculated by normalizing levels of scission of the unmodified oligonucleotide (None) to 1. Error bars represent the standard deviations of three independent experiments.

ϵdA lesions are strong topoisomerase II poisons and stimulate DNA cleavage ∼8−10-fold (*21*), initial studies focused on ϵdC and ϵdG.

Etheno adducts are generated in the cell by two major pathways. First, they are formed by exposure of DNA to *trans*-4,5-epoxy-2(*E*)-decenal, which is a byproduct of endogenous lipid peroxidation (*51*). Second, they are formed by environmental exposure to a known carcinogen, vinyl chloride, and related compounds such as urethane (*52*). As seen in Figure 2, ϵdC adducts were strong position-specific poisons of topoisomerase IIα. When located between the scissile bonds at the +2 and +3 positions, respectively, these adducts stimulated DNA cleavage mediated by human topoisomerase IIα ∼6- and 12-fold. Although the +2 etheno adduct did not have as pronounced an effect on DNA cleavage as did an apyrimidinic site at the +2 position, cleavage stimulation by the +3 ϵdC was greater (12-fold). Moreover, as reported for abasic sites, ϵdC adducts that were located immediately outside of the scissile bonds (at the −3 or +6 positions) inhibited enzyme-mediated DNA scission (Figure 2).

Sugar-ring modifications are modest topoisomerase II poisons (*19*, *20*, *53*). For example, inclusion of ribonucleotides between the scissile bonds stimulates DNA cleavage up to ∼2-fold (see inset, Figure 2). To determine whether the effects of base- and sugar-ring modifications on topoisomerase II are synergistic, the ϵdC adducts at the +2 and +3 positions were replaced with ϵrC. Little additional cleavage enhancement was observed at the +2 position, and a modest increase was seen at the +3 position. Therefore, the effects of the two modifications, while potentially additive, do not appear to be synergistic.

The effects of ϵdG on DNA cleavage mediated by human topoisomerase IIα also were examined (Figure 3). ϵdG was a moderate to strong topoisomerase II poison and stimulated DNA cleavage ∼6- and 5-fold when substituted at the +2 and +3 positions, respectively. Similar to results with ϵdC, the presence of an ϵdG adduct at the +2 position had a smaller effect on DNA cleavage than the corresponding abasic (apurinic) site but had a larger effect than the abasic site when present at the +3 position. Once again, the presence of an adduct located outside of the scissile bonds at the −3 position did not enhance DNA scission by the enzyme (Figure 3).

Since the etheno adducts all stimulated DNA scission mediated by human topoisomerase IIα, the effects of the structurally related exocyclic lesion, M_1dG (see Figure 1), on DNA cleavage were examined. M_1dG is a naturally occurring exocyclic adduct detected in human DNA (*54*−*57*). It is formed by reaction of DNA with malondialdehyde, a byproduct of thromboxane biosynthesis and a product of oxidative degradation of polyunsaturated lipids, and other base propenals (*58*).

M_1dG stimulated DNA cleavage ∼7−8-fold, which was slightly higher than values observed for ϵdG (Figure 3). There is a caveat to these results, however. In some sequences,

when M_1dG is located directly across from a cytosine, the exocyclic ring opens (see Figure 1) (*59*, *60*). In contrast to M_1dG, the ring-opened form [i.e., N^2-(3-oxo-1-propenyl)-deoxyguanosine] induces minimal distortion into the DNA backbone (*60*). To further explore this issue, the effects of M_1dG on topoisomerase II-mediated DNA cleavage were examined when the bases across from these adducts were changed to thymine residues (Figure 3, inset). M_1dG has been observed in a ring-closed structure when situated across from a thymine (*59*). Results were similar to those obtained with cytosine-containing sequences. Two possible conclusions can be drawn from these findings. Either M_1dG is a strong topoisomerase II poison in both the ring-opened and ring-closed forms or the adduct remains in the ring-closed form in both of the oligonucleotide substrates.

Due to the uncertainty regarding the exocyclic structure of M_1dG, the effects of PdG on topoisomerase II-mediated DNA cleavage were characterized. Although PdG is not a naturally occurring DNA adduct, it has been used extensively as a stable model for the ring-closed form of M_1dG (*61*−*63*). It is structurally simular to M_1dG, except that the exocyclic ring is aliphatic rather than aromatic (see Figure 1). In addition, the exocyclic ring of PdG never opens (*61*−*63*). Results for PdG are shown in Figure 3. DNA cleavage enhancement was identical to that observed for M_1dG. On the basis of these data, we suggest that M_1dG exists in a closed-ring form in the DNA sequence used for the present study. Together with the results of the etheno base adducts, these findings indicate that a variety of exocyclic base adducts are position-specific poisons of human topoisomerase IIα.

*Effects of Exocyclic Base Adducts on DNA Religation Mediated by Human Topoisomerase II*α. Topoisomerase II poisons can increase levels of enzyme−DNA cleavage complexes by opposite mechanisms that are not mutually exclusive. Some anticancer drugs, such as etoposide, strongly inhibit the ability of topoisomerase II to ligate cleaved DNA molecules (*28*−*30*, *64*). In contrast, other drugs such as the quinolone CP-115 953 have little effect on rates of ligation and presumably raise the concentration of cleavage complexes by increasing the overall rate of DNA cleavage (*28*−*30*, *65*). This latter rate reflects both the binding constant of topoisomerase II for its nucleic acid substrate (i.e., the formation of the noncovalent enzyme−DNA complex) and the forward rate of the chemical DNA scission event.

Previous studies indicate that a variety of DNA lesions act on topoisomerase II by the latter mechanism (*15*−*19*, *21*, *66*). To determine if this was the case for the exocyclic base adducts used in the present work, the effects of ϵdC, ϵrC, ϵdG, M_1dG, and PdG on the ability of human topoisomerase IIα to religate DNA were determined. Results for lesions located at the +3 position are shown in Figure 4 and Table 1. The apparent first-order rate of religation for the unmodified sequence was 0.055 s^{-1}. In all cases, religation rates were higher for oligonucleotide substrates that contained adducts. Similar results were obtained when adducts were located at the +2 position (not shown). The fastest rate obtained (0.138 s^{-1} for ϵrC) was ∼2.5-fold higher than seen with the parental unmodified substrate. These results support previous findings and indicate that exocyclic base adducts do not increase levels of topoisomerase II−DNA cleavage complexes by inhibiting enzyme-mediated DNA ligation.

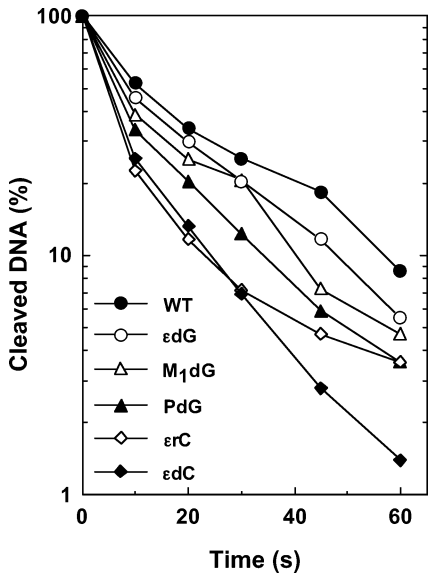

FIGURE 4: Alkylated DNA bases do not inhibit DNA religation mediated by human topoisomerase IIα. The effects of DNA adducts at the +3 position of the top strand on DNA religation mediated by topoisomerase IIα are shown. Oligonucleotide substrates are as in Figures 2 and 3. DNA religation was monitored on the strand opposite the lesions. The amount of DNA cleavage observed at equilibrium for each substrate was set to 100% at time zero. DNA religation was quantified by the loss of cleaved molecules. Data represent the average of three independent experiments.

Table 1: Apparent First-Order Rates of Religation of Damage-Containing Oligonucleotides by Human Topoisomerase IIα[a]

DNA lesion	rate (s^{-1})	DNA lesion	rate (s^{-1})
wild type	0.055	PdG	0.115
ϵdG	0.077	ϵdC	0.125
M_1dG	0.099	ϵrC	0.138

[a] DNA lesions were located at the +3 position as shown in Figures 2 and 3.

*Effects of Exocyclic Base Adducts on Topoisomerase II*α*−DNA Binding.* The above results suggest that exocyclic base adducts raise levels of enzyme−DNA cleavage complexes by increasing the overall rate of cleavage complex formation (which reflects both the enzyme−DNA binding constant and the actual rate of DNA scission). At the present time, it is not possible to determine directly the rate of the DNA scission event. Therefore, to further clarify the mechanistic basis for the increased cleavage of damaged DNA, the effects of lesions on the ability of human topoisomerase IIα to bind its nucleic acid substrate were determined.

A competitive nitrocellulose DNA filter-binding assay was employed. The human enzyme was incubated with a mixture of radiolabeled unmodified oligonucleotide and a nonlabeled competitor oligonucleotide that contained no damage, an abasic site, or ϵdC, ϵdG, M_1dG, PdG, or rC at the +2 position. Selected competition curves are shown in Figure 5, and IC_{50} values for all of the oligonucleotides are given in Table 2. IC_{50} values for the undamaged and damaged substrates were similar and ranged from 78 to 115 nM. These results indicate that the increased levels of topoisomerase II-mediated DNA cleavage observed with substrates that contain exocyclic base adducts (at least at the +2 position) are not due to an increased binding affinity of the human enzyme for damaged DNA. Together with the present and

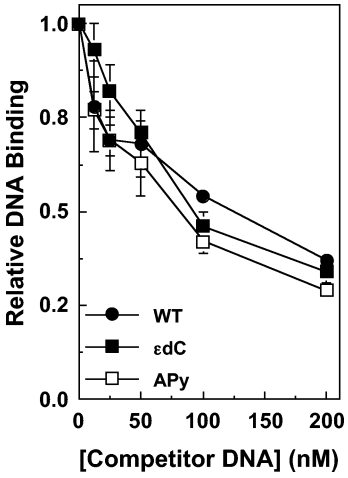

FIGURE 5: The presence of exocyclic DNA adducts does not affect the binding affinity of human topoisomerase IIα for DNA. Results from a competitive nitrocellulose filter-binding assay are shown. Oligonucleotide substrates are as in Figures 2 and 3. ^{32}P-labeled unmodified oligonucleotide (50 nM) was incubated with topoisomerase IIα and increasing concentrations of unmodified (WT) oligonucleotide or substrates containing an apyrimidinic site (APy) or an ϵdC adduct at the +2 position of the top strand. Error bars represent the standard deviations of three independent experiments.

Table 2: Topoisomerase IIα−DNA Nitrocellulose Filter-Binding Competition Assays[a]

DNA lesion	IC_{50} (nM) ± SD	DNA lesion	IC_{50} (nM) ± SD
wild type	115 ± 13	ϵdG	96 ± 4
APy	78 ± 9	M_1dG	103 ± 15
APu	104 ± 13	PdG	114 ± 16
ϵdC	100 ± 7	rC	92 ± 13

[a] DNA lesions were located at the +2 position as shown in Figures 2 and 3. IC_{50} values represent the concentration of damage-containing oligonucleotides required to decrease the binding of radiolabeled unmodified oligonucleotide (50 nM) to human topoisomerase IIα (220 nM) by 50%. Values are the averages of three independent experiments with their respective standard deviations.

previous (*15*−*19*, *21*, *66*) religation data, these findings suggest that the forward rate of the DNA scission event catalyzed by topoisomerase IIα is accelerated when damaged bases are located between the scissile bonds.

Enhancement of DNA Cleavage Mediated by Topoisomerase IIα in Cultured Human Cells Treated with 2-Chloroacetaldehyde. The cellular consequences of any given DNA lesion are determined by the proteins that first interact with it (*6*). For example, if a glycosylase encounters an alkylated base, it is likely that the DNA damage will be converted into an abasic site and channeled into the BER pathway (*8*). However, if a DNA polymerase attempts to traverse that same lesion, the damage may trigger recombination pathways or be fixed in the genome as a permanent mutation (*4*, *5*, *7*, *67*, *68*).

Previous studies indicate that some DNA lesions increase levels of topoisomerase I−DNA cleavage complexes in cultured cells (*13*, *43*). This finding implies that if topoisomerase I encounters a DNA adduct, the original damage can lead to the generation of protein-linked single-stranded DNA breaks. Thus, the actions of topoisomerases have the potential to dramatically alter the genotoxicity of specific forms of DNA damage.

All of the in vitro data suggest that type II topoisomerases, like their type I counterparts, should interact with DNA

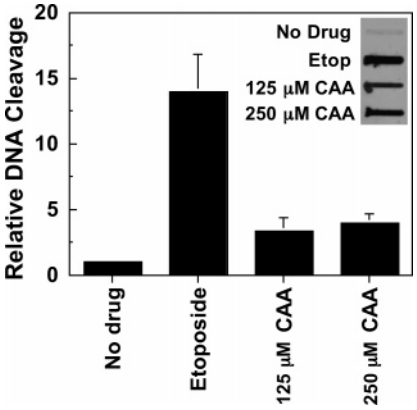

FIGURE 6: 2-Chloroacetaldehyde induces topoisomerase IIα-mediated DNA cleavage in cultured human CEM cells. The ICE bioassay was used to monitor levels of enzyme−DNA cleavage complexes. Cells were exposed to 50 μM etoposide (Etop) for 1 h or to 125 or 250 μM 2-chloroacetaldehyde (CAA) for 4 h. Cells were lysed and pelleted through a CsCl cushion to separate topoisomerase IIα−DNA cleavage complexes from free enzyme. Pellets were slot-blotted and probed for human topoisomerase IIα. A typical slot-blot is shown in the inset. Relative DNA cleavage was calculated by normalizing levels of scission in untreated cultures (No Drug) to 1. Error bars represent the standard deviations of four independent experiments.

lesions in the cell. However, physiological encounters between topoisomerase II and DNA damage have yet to be demonstrated. Therefore, the effects of 2-chloroacetaldehyde on levels of DNA cleavage mediated by topoisomerase IIα were determined in cultured human cells. 2-Chloroacetaldehyde is one of the reactive products of vinyl chloride metabolism and induces etheno base adducts, such as ϵdG, ϵdC, and ϵdA, in treated cells (*44*, *69*, *70*).

As determined using the ICE bioassay, treatment of human CEM leukemia cells with 125 or 250 μM 2-chloroacetaldehyde increased levels of topoisomerase IIα-mediated DNA cleavage ∼3.3- or 3.9-fold, respectively (Figure 6). Although substantial, this increase is less than observed following treatment of cells with 50 μM etoposide. However, it should be noted that etoposide increases levels of topoisomerase II-linked DNA breaks through a direct interaction with the enzyme. In contrast, 2-chloroacetaldehyde acts through the generation of DNA lesions.[2] While the cellular concentration of base adducts induced by the DNA damaging agent is not known, it is highly likely that it is significantly lower than the initial concentration (125 or 250 μM) of 2-chloroacetaldehyde used to treat cells.

Increases in DNA scission mediated by human topoisomerase IIα also were observed when MDA MB 231 or MCF-7 breast cancer cells were treated with 2-chloroacetaldehyde (not shown). Levels of cleavage rose ∼1.8- and 2.5-fold, or ∼2.3- and 4.8-fold when these two cell lines were treated with 125 or 250 μM 2-chloroacetaldehyde, respectively.

Since 2-chloroacetaldehyde is a bifunctional alkylating agent, it is possible that the increase in covalent topo-

[2] It should be noted that 250 μM 2-chloroacetaldehyde in a plasmid-based topoisomerase II−DNA cleavage assay (*65*) did not enhance levels of enzyme-mediated DNA scission (not shown). Under the conditions employed, significantly less than one adduct per plasmid should have been generated by the presence of this chemical (*45*). Therefore, it is concluded that 2-chloroacetaldehyde does not act directly as a topoisomerase II poison.

isomerase IIα−DNA complexes seen in Figure 6 actually represents enzyme−DNA cross-links rather than enzyme-mediated scission. However, we do not believe that this is the case. The concentrations of 2-chloroacetaldehyde used for these studies, 125 and 250 μM, are ∼3 orders of magnitude lower than that normally used to induce protein−DNA cross-links with more reactive agents such as formaldehyde. Moreover, a previous study demonstrated that treatment of human Burkitt's lymphoma cells with 750 μM 2-chloroacetaldehyde did not significantly increase levels of protein−DNA cross-links (*71*).

To further address this issue, three control experiments were carried out (not shown). First, no protein−DNA cross-links were observed when topoisomerase IIα was incubated with plasmid molecules and 250 μM 2-chloroacetaldehyde. Second, no p53−DNA cross-links were observed in CEM cells following exposure to 250 μM 2-chloroacetaldehyde. Finally, treatment of cultured human cells with methyl methanesulfonate, a monofunctional alkylating agent that cannot generate protein−DNA cross-links, increased levels of covalent topoisomerase IIα−DNA complexes (unpublished results).

Taken together, the data presented above provide strong evidence that topoisomerase IIα interacts with DNA damage in cultured human cells and that this interaction increases levels of DNA cleavage mediated by the enzyme.

DISCUSSION

The presence of base adducts in the genetic material can lead to mutations because replicative and repair DNA polymerases often insert wrong nucleotides opposite of adducted bases (*3*, *67*, *72*). In addition, lesions can lead to chromosomal aberrations such as insertions, deletions, rearrangements, and translocations (*67*, *73*, *74*). Many of these latter effects are attributed to recombination pathways that are triggered when lesions stall DNA replication forks (*4*, *7*, *68*). However, similar chromosomal aberrations are observed following treatment of cells with topoisomerase II poisons (*29*, *31*−*33*).

Topoisomerase II is an essential enzyme that removes knots and tangles from the genetic material (*30*, *75*). However, because the enzyme generates a protein-linked double-stranded DNA break as a requisite step in its catalytic cycle, topoisomerase II also has the potential to fragment the genome every time it functions (*30*, *75*−*78*). As a result of this latter property of topoisomerase II, this enzyme plays a central role in cancer. A number of drugs that act as topoisomerase II poisons are used as front-line chemotherapeutic agents for the treatment of human malignancies. Conversely, evidence indicates that under some circumstances, exposure to topoisomerase II poisons (drugs, natural products, or environmental pollutants) can lead to the generation of specific leukemias.

In addition to chemicals that increase levels of topoisomerase II-mediated DNA cleavage, several DNA lesions have been found to poison the type II enzyme in vitro (*15*−*19*). For example, when located within the four-base stagger that separates the scissile bonds of a topoisomerase II DNA cleavage site, abasic sites increase enzyme-mediated scission as much as 10−20-fold (*15*−*18*, *21*). Previous studies from this laboratory suggest that the ability to distort the double helix contributes to the potential of a lesion to act as a topoisomerase II poison (*18*, *21*). Therefore, to further characterize the range of DNA lesions that alter enzyme function, the effects of bulky exocyclic adducts on the DNA cleavage activity of human topoisomerase IIα were assessed.

ϵdC, ϵdG, M_1dG, and PdG all were moderate to strong position-specific topoisomerase IIα poisons. When located in the +2 or +3 positions between the scissile bonds, they increased levels of DNA cleavage ∼5−17-fold. Enhanced cleavage did not appear to result from an increased affinity of topoisomerase IIα for the adducted DNA or a decreased rate of enzyme-mediated religation. Therefore, it is concluded that these lesions acted as topoisomerase II poisons by accelerating the forward rate of enzyme-mediated DNA scission. This conclusion is consistent with prior studies on DNA lesions (*15*−*19*, *21*) and evidence that suggests that the recognition of DNA cleavage sites by topoisomerase II is governed by chemical steps rather than by the enzyme−DNA binding step (*64*, *79*, *80*).

The mechanistic basis for the enhancement of topoisomerase II-mediated DNA scission by DNA adducts is not understood. However, it is believed that they act by distorting the double helix (*18*, *21*, *29*, *65*). When this distortion is located between the two scissile bonds of a topoisomerase II DNA cleavage site, it facilitates interactions within the active site of the enzyme and accelerates rates of scission. However, when it is located immediately outside of the scissile bonds, the DNA distortion presumably alters the alignment of the cleavage sequence within the active site and decreases scission. It is notable that ϵdC, M_1dG, and PdG, adducts for which structural data are available, introduce kinks into DNA (*59*, *60*, *62*, *81*). All of these adducts project their exocyclic ring into the major groove of the double helix and disrupt base pairing (*59*, *60*, *62*, *81*). Beyond these general features, it is difficult to compare the precise alterations in DNA structure that are induced by ϵdC, M_1dG, and PdG, as the structural data for each were generated within different DNA sequence contexts.

It has been known for nearly a decade that some DNA lesions (primarily abasic sites) could increase topoisomerase II-mediated DNA cleavage in vitro (*15*−*19*, *21*). However, it had never been demonstrated that lesions act as topoisomerase II poisons in living systems. Results of the present study indicate that levels of DNA cleavage mediated by topoisomerase IIα rise substantially when cultured human cells are exposed to 2-chloroacetaldehyde, a chemical reagent that induces the formation of etheno−base adducts in vivo (*44*, *45*, *70*). On the basis of this finding, we believe that DNA lesions are capable of acting as physiological poisons of topoisomerase II.

The first step of BER, the pathway that repairs etheno adducts, is the conversion of alkylated bases to abasic sites by a DNA glycosylase (*82*, *83*). Since abasic sites are strong topoisomerase II poisons, it is not known whether increased DNA cleavage by topoisomerase IIα ultimately results from a direct interaction with the etheno adducts or rather with the resulting abasic sites, or a combination of both. It has been proposed that proteins in the BER pathway act in a cooperative fashion, with each handing the processed repair intermediate to the subsequent protein in the pathway (*84*). Although a variety of BER intermediates are topoisomerase II poisons in vitro (*50*), it has yet to be determined whether

the enzyme can successfully compete with APE1 or DNA polymerase β for access to abasic sites generated by this repair pathway in vivo. This important issue remains the subject of future investigation.

Results of the present study indicate that DNA lesions have the potential to act as topoisomerase II poisons in cultured human cells. The physiological ramifications of this finding are not known. Formation of a topoisomerase II−DNA cleavage complex proximal to the site of a DNA adduct provides no obvious advantage for the repair of that adduct. If anything, generation of a protein-associated double-stranded break adjacent to a DNA lesion would diminish the chances of successful repair and greatly increase the likelihood of mutagenesis or chromosomal rearrangements. In this regard, topoisomerase II-mediated DNA cleavage has been implicated in initiating specific types of leukemia (*36*−*38*). These leukemias generally display chromosomal translocations with breakpoints that originate within the *MLL* gene at chromosomal band 11q23 (*36*−*42*). Translocations involving chromosomal band 11q23 are observed in cancer patients treated with drug regimens that include topoisomerase II poisons (*40*−*42*, *85*, *86*). They also are observed in infant leukemias and are associated with the ingestion of naturally occurring topoisomerase II poisons (*36*−*42*). Thus, interactions of topoisomerase II with DNA lesions may be deleterious in nature and may simply be the price that the cell has to pay to have an enzyme that can pass one DNA double helix through another.

Conversely, if the genetic material of a cell sustains sufficiently high levels of damage, the resulting increase in topoisomerase II-mediated DNA cleavage may help to trigger apoptotic pathways (*29*, *87*, *88*) that remove the damaged cell from the population. In this case, interactions of topoisomerase II with DNA lesions could play a positive role in the survival of an organism. Whether the cellular recognition of DNA damage by topoisomerase II ultimately is demonstrated to have a negative or a positive physiological effect (or both), it is clear that this interaction provides an additional layer of complexity that the cell has to cope with in order to maintain the integrity of its genome.

ACKNOWLEDGMENT

We are grateful to Dr. Susan D. Cline for helpful discussions, to A. Kathleen McClendon for providing the human topoisomerase IIα preparation used for DNA filter-binding assays, to Angela Brock, Ivan Korzelaov, and Karen Angel for the synthesis and purification of the ϵdG oligo at the −3 position, and to Erin L. Baldwin and Jennifer S. Dickey for critical reading of the manuscript.

REFERENCES

1. Xiao, W., and Samson, L. (1993) In vivo evidence for endogenous DNA alkylation damage as a source of spontaneous mutation in eukaryotic cells, *Proc. Natl. Acad. Sci. U.S.A. 90*, 2117−2121.
2. Marnett, L. J. (2002) Oxy radicals, lipid peroxidation and DNA damage, *Toxicology 181*−*182*, 219−222.
3. Nair, J., Barbin, A., Velic, I., and Bartsch, H. (1999) Etheno DNA-base adducts from endogenous reactive species, *Mutat. Res. 424*, 59−69.
4. Guengerich, F. P., Langouet, S., Mican, A. N., Akasaka, S., Muller, M., and Persmark, M. (1999) Formation of etheno adducts and their effects on DNA polymerases, *IARC Sci. Publ.*, 137−145.
5. Yu, S. L., Lee, S. K., Johnson, R. E., Prakash, L., and Prakash, S. (2003) The stalling of transcription at abasic sites is highly mutagenic, *Mol. Cell. Biol. 23*, 382−388.
6. Cline, S. D., and Hanawalt, P. C. (2003) Who's on first in the cellular response to DNA damage?, *Nat. Rev. Mol. Cell. Biol. 4*, 361−372.
7. Cline, S. D., Riggins, J. N., Tornaletti, S., Marnett, L. J., and Hanawalt, P. C. (2004) Malondialdehyde adducts in DNA arrest transcription by T7 RNA polymerase and mammalian RNA polymerase II, *Proc. Natl. Acad. Sci. U.S.A. 101*, 7275−7280.
8. Barnes, D. E., and Lindahl, T. (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells, *Annu. Rev. Genet. 38*, 445−476.
9. Pourquier, P., Bjornsti, M. A., and Pommier, Y. (1998) Induction of topoisomerase I cleavage complexes by the vinyl chloride adduct 1,N^6-ethenoadenine, *J. Biol. Chem. 273*, 27 245−27 249.
10. Pourquier, P., Ueng, L. M., Fertala, J., Wang, D., Park, H. J., Essigmann, J. M., Bjornsti, M. A., and Pommier, Y. (1999) Induction of reversible complexes between eukaryotic DNA topoisomerase I and DNA-containing oxidative base damages: 7,8-dihydro-8-oxoguanine and 5-hydroxycytosine, *J. Biol. Chem. 274*, 8516−8523.
11. Pommier, Y., Laco, G. S., Kohlhagen, G., Sayer, J. M., Kroth, H., and Jerina, D. M. (2000) Position-specific trapping of topoisomerase I-DNA cleavage complexes by intercalated benzo-[*a*]pyrene diol epoxide adducts at the 6-amino group of adenine, *Proc. Natl. Acad. Sci. U.S.A. 97*, 10 739−10 744.
12. Pommier, Y., Kohlhagen, G., Pourquier, P., Sayer, J. M., Kroth, H., and Jerina, D. M. (2000) Benzo[*a*]pyrene diol epoxide adducts in DNA are potent suppressors of a normal topoisomerase I cleavage site and powerful inducers of other topoisomerase I cleavages, *Proc. Natl. Acad. Sci. U.S.A. 97*, 2040−2045.
13. Pourquier, P., Takebayashi, Y., Urasaki, Y., Gioffre, C., Kohlhagen, G., and Pommier, Y. (2000) Induction of topoisomerase I cleavage complexes by 1-beta-D-arabinofuranosylcytosine (ara-C) in vitro and in ara-C-treated cells, *Proc. Natl. Acad. Sci. U.S.A. 97*, 1885−1890.
14. Pourquier, P., Ueng, L. M., Kohlhagen, G., Mazumder, A., Gupta, M., Kohn, K. W., and Pommier, Y. (1997) Effects of uracil incorporation, DNA mismatches, and abasic sites on cleavage and religation activities of mammalian topoisomerase I, *J. Biol. Chem. 272*, 7792−7796.
15. Kingma, P. S., Corbett, A. H., Burcham, P. C., Marnett, L. J., and Osheroff, N. (1995) Abasic sites stimulate double-stranded DNA cleavage mediated by topoisomerase II: anticancer drugs mimic endogenous DNA lesions, *J. Biol. Chem. 270*, 21441−21444.
16. Kingma, P. S., and Osheroff, N. (1997) Spontaneous DNA damage stimulates topoisomerase II-mediated DNA cleavage, *J. Biol. Chem. 272*, 7488−7493.
17. Kingma, P. S., Greider, C. A., and Osheroff, N. (1997) Spontaneous DNA lesions poison human topoisomerase IIα and stimulate cleavage proximal to leukemic 11q23 chromosomal breakpoints, *Biochemistry 36*, 5934−5939.
18. Kingma, P. S., and Osheroff, N. (1997) Apurinic sites are position-specific topoisomerase II poisons, *J. Biol. Chem. 272*, 1148−1155.
19. Cline, S. D., and Osheroff, N. (1999) Cytosine arabinoside (araC) lesions are position-specific topoisomerase II poisons and stimulate DNA cleavage mediated by the human type II enzymes, *J. Biol. Chem. 274*, 29740−29743.
20. Wang, Y., Thyssen, A., Westergaard, O., and Andersen, A. H. (2000) Position-specific effect of ribonucleotides on the cleavage activity of human topoisomerase II, *Nucleic Acids Res. 28*, 4815−4821.
21. Sabourin, M., and Osheroff, N. (2000) Sensitivity of human type II topoisomerases to DNA damage: stimulation of enzyme-mediated DNA cleavage by abasic, oxidized and alkylated lesions, *Nucleic Acids Res. 28*, 1947−1954.
22. Wang, J. C. (1996) DNA topoisomerases, *Annu. Rev. Biochem. 65*, 635−692.
23. Champoux, J. J. (2001) DNA topoisomerases: structure, function, and mechanism, *Annu. Rev. Biochem. 70*, 369−413.
24. Nitiss, J. L. (1998) Investigating the biological functions of DNA topoisomerases in eukaryotic cells, *Biochim. Biophys. Acta 1400*, 63−81.
25. Wang, J. C. (2002) Cellular roles of DNA topoisomerases: a molecular perspective, *Nat. Rev. Mol. Cell. Biol. 3*, 430−440.

26. Wang, J. C. (1998) Moving one DNA double helix through another by a type II DNA topoismerase: the story of a simple molecular machine, *Q. Rev. Biophys. 31*, 107−144.
27. Berger, J. M. (1998) Type II DNA topoisomerases, *Curr. Opin. Struct. Biol. 8*, 26−32.
28. 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.
29. Wilstermann, A. M., and Osheroff, N. (2003) Stabilization of eukaryotic topoisomerase II-DNA cleavage complexes, *Curr. Top. Med. Chem. 3*, 321−338.
30. Burden, D. A., and Osheroff, N. (1998) Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme, *Biochim. Biophys. Acta 1400*, 139−154.
31. Pommier, Y. (1997) in *Cancer Drug Discovery and Development* (Teicher, B. A., Ed.) pp 153−174, Humana Press, Totowa, NJ.
32. Kaufmann, S. H. (1998) Cell death induced by topoisomerase-targeted drugs: more questions than answers, *Biochim. Biophys. Acta 1400*, 195−211.
33. Capranico, G., Giaccone, G., and D'Incalci, M. (1999) DNA topoisomerase II poisons and inhibitors, *Cancer Chemother. Biol. Response Modif. 18*, 125−143.
34. Reddy, K. (2004) Evolving role of topotecan in treating small-cell lung cancer, *Clin. Lung Cancer 5*, 270−272.
35. Toonen, T. R., and Hande, K. R. (2001) Topoisomerase II inhibitors, *Cancer Chemother. Biol. Response Modif. 19*, 129−147.
36. 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.
37. 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.
38. 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.
39. Felix, C. A., Lange, B. J., Hosler, M. R., Fertala, J., and Bjornsti, M.-A. (1995) Chromosome band 11q23 translocation breakpoints are DNA topoisomerase II cleavage sites, *Cancer Res. 55*, 4287−4292.
40. Felix, C. A., Hosler, M. R., Winick, N. J., Masterson, M., Wilson, A. E., and Lange, B. J. (1995) ALL-1 gene rearrangements in DNA topoisomerase II inhibitor-related leukemia in children, *Blood 85*, 3250−3256.
41. Felix, C. A., and Lange, B. J. (1999) Leukemia in infants, *Oncologist 4*, 225−240.
42. Rowley, J. D. (2001) Chromosome translocations: dangerous liaisons revisited, *Nat. Rev. Cancer 1*, 245−250.
43. Pourquier, P., Waltman, J. L., Urasaki, Y., Loktionova, N. A., Pegg, A. E., Nitiss, J. L., and Pommier, Y. (2001) Topoisomerase I-mediated cytotoxicity of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine: trapping of topoisomerase I by the O^6-methylguanine, *Cancer Res. 61*, 53−58.
44. Zielinski, B., and Hergenhahn, M. (2001) 2-Chloroacetaldehyde induces epsilondA DNA adducts in DNA of Raji cells as demonstrated by an improved HPLC-fluorimetry method, *Fresenius' J. Anal. Chem. 370*, 97−100.
45. Guengerich, F. P. (1992) Roles of the vinyl chloride oxidation products 1-chlorooxirane and 2-chloroacetaldehyde in the in vitro formation of etheno adducts of nucleic acid bases, *Chem. Res. Toxicol. 5*, 2−5.
46. Schnetz-Boutaud, N. C., Mao, H., Stone, M. P., and Marnett, L. J. (2000) Synthesis of oligonucleotides containing the alkali-labile pyrimidopurinone adduct, M(1)G, *Chem. Res. Toxicol. 13*, 90−95.
47. Kouchakdjian, M., Marinelli, E., Gao, X. L., Johnson, F., Grollman, A., and Patel, D. (1989) NMR studies of exocyclic 1,N^2-propanodeoxyguanosine adducts (X) opposite purines in DNA duplexes: protonated X(syn)·A(anti) pairing (acidic pH) and X(syn)·G(anti) pairing (neutral pH) at the lesion site, *Biochemistry 28*, 5647−5657.
48. Marinelli, E. R., Johnson, F., Iden, C. R., and Yu, P. L. (1990) Synthesis of 1,N^2-(1,3-propano)-2′-deoxyguanosine and incorporation into oligodeoxynucleotides: a model for exocyclic acrolein-DNA adducts, *Chem. Res. Toxicol. 3*, 49−58.
49. Saparbaev, M., Langouet, S., Privezentzev, C. V., Guengerich, F. P., Cai, H., Elder, R. H., and Laval, J. (2002) 1,N^2-ethenoguanine, a mutagenic DNA adduct, is a primary substrate of *Escherichia coli* mismatch-specific uracil-DNA glycosylase and human alkylpurine-DNA-*N*-glycosylase, *J. Biol. Chem. 277*, 26987−26993.
50. Wilstermann, A. M., and Osheroff, N. (2001) Base excision repair intermediates as topoisomerase II poisons, *J. Biol. Chem. 276*, 46290−46296.
51. Lee, S. H., Oe, T., and Blair, I. A. (2002) 4,5-Epoxy-2(*E*)-decenal-induced formation of 1,N^6-etheno-2′-deoxyadenosine and 1,N^2-etheno-2′-deoxyguanosine adducts, *Chem. Res. Toxicol. 15*, 300−304.
52. Swenberg, J. A., Fedtke, N., Ciroussel, F., Barbin, A., and Bartsch, H. (1992) Etheno adducts formed in DNA of vinyl chloride-exposed rats are highly persistent in liver, *Carcinogenesis 13*, 727−729.
53. Wang, Y., Knudsen, B. R., Bjergbaek, L., Westergaard, O., and Andersen, A. H. (1999) Stimulated activity of human topoisomerases IIalpha and IIbeta on RNA-containing substrates, *J. Biol. Chem. 274*, 22839−22846.
54. Rouzer, C. A., Chaudhary, A. K., Nokubo, M., Ferguson, D. M., Reddy, G. R., Blair, I. A., and Marnett, L. J. (1997) Analysis of the malondialdehyde-2′-deoxyguanosine adduct pyrimidopurinone in human leukocyte DNA by gas chromatography/electron capture/negative chemical ionization/mass spectrometry, *Chem. Res. Toxicol. 10*, 181−188.
55. Fang, J. L., Vaca, C. E., Valsta, L. M., and Mutanen, M. (1996) Determination of DNA adducts of malonaldehyde in humans: effects of dietary fatty acid composition, *Carcinogenesis 17*, 1035−1040.
56. Vaca, C. E., Fang, J. L., Mutanen, M., and Valsta, L. (1995) ^{32}P-postlabelling determination of DNA adducts of malonaldehyde in humans: total white blood cells and breast tissue, *Carcinogenesis 16*, 1847−1851.
57. Chaudhary, A. K., Nokubo, G., Reddy, R., Yeola, S. N., Morrow, J. D., Blair, I. A., and Marnett, L. J. (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver, *Science 265*, 1580−1582.
58. Marnett, L. J. (1999) Lipid peroxidation-DNA damage by malondialdehyde, *Mutat. Res. 424*, 83−95.
59. Mao, H., Schnetz-Boutaud, N. C., Weisenseel, J. P., Marnett, L. J., and Stone, M. P. (1999) Duplex DNA catalyzes the chemical rearrangement of a malondialdehyde deoxyguanosine adduct, *Proc. Natl. Acad. Sci. U.S.A. 96*, 6615−6620.
60. Mao, H., Reddy, G. R., Marnett, L. J., and Stone, M. P. (1999) Solution structure of an oligodeoxynucleotide containing the malondialdehyde deoxyguanosine adduct N^2-(3-oxo-1-propenyl)-dG (ring-opened M1G) positioned in a (CpG)3 frameshift hotspot of the *Salmonella typhimurium* hisD3052 gene, *Biochemistry 38*, 13491−13501.
61. Singh, U. S., Moe, J. G., Reddy, G. R., Weisenseel, J. P., Marnett, L. J., and Stone, M. P. (1993) ^{1}H NMR of an oligodeoxynucleotide containing a propanodeoxyguanosine adduct positioned in a (CG)3 frameshift hotspot of *Salmonella typhimurium* hisD3052: Hoogsteen base-pairing at pH 5.8, *Chem. Res. Toxicol. 6*, 825−836.
62. Weisenseel, J. P., Moe, J. G., Reddy, G. R., Marnett, L. J., and Stone, M. P. (1995) Structure of a duplex oligodeoxynucleotide containing propanodeoxyguanosine opposite a two-base deletion in the (CpG)3 frame shift hotspot of *Salmonella typhimurium* hisD3052 determined by ^{1}H NMR and restrained molecular dynamics, *Biochemistry 34*, 50−64.
63. Weisenseel, J. P., Reddy, G. R., Marnett, L. J., and Stone, M. P. (2002) Structure of the 1,*N*(2)-propanodeoxyguanosine adduct in a three-base DNA hairpin loop derived from a palindrome in the *Salmonella typhimurium* hisD3052 gene, *Chem. Res. Toxicol. 15*, 140−152.
64. Bromberg, K. D., Burgin, A. B., and Osheroff, N. (2003) A two drug model for etoposide action against human topoisomerase IIα, *J. Biol. Chem. 278*, 7406−7412.
65. Bromberg, K. D., Burgin, A. B., and Osheroff, N. (2003) Quinolone action against human topoisomerase IIalpha: stimulation of enzyme-mediated double-stranded DNA cleavage, *Biochemistry 42*, 3393−3398.
66. Kingma, P. S., and Osheroff, N. (1998) Topoisomerase II-mediated DNA cleavage and religation in the absence of base pairing: abasic lesions as a tool to dissect enzyme mechanism, *J. Biol. Chem. 273*, 17999−18002.

67. Barbin, A. (2000) Etheno-adduct-forming chemicals: from mutagenicity testing to tumor mutation spectra, *Mutat. Res. 462*, 55−69.
68. Chen, Y. H., and Bogenhagen, D. F. (1993) Effects of DNA lesions on transcription elongation by T7 RNA polymerase, *J. Biol. Chem. 268*, 5849−5855.
69. Barbin, A., Bartsch, H., Leconte, P., and Radman, M. (1981) Studies on the miscoding properties of 1,N^6-ethenoadenine and 3,N^4-ethenocytosine, DNA reaction products of vinyl chloride metabolites, during in vitro DNA synthesis, *Nucleic Acids Res. 9*, 375−387.
70. Barbin, A., Friesen, M., O'Neill, I. K., Croisy, A., and Bartsch, H. (1986) New adducts of chloroethylene oxide and chloroacetaldehyde with pyrimidine nucleosides, *Chem.-Biol. Interact. 59*, 43−54.
71. Costa, M., Zhitkovich, A., Harris, M., Paustenbach, D., and Gargas, M. (1997) DNA-protein cross-links produced by various chemicals in cultured human lymphoma cells, *J. Toxicol. Environ. Health 50*, 433−449.
72. Barbin, A. (1998) Formation of DNA etheno adducts in rodents and humans and their role in carcinogenesis, *Acta Biochim. Pol. 45*, 145−161.
73. Giri, A. K. (1995) Genetic toxicology of vinyl chloride—a review, *Mutat. Res. 339*, 1−14.
74. Barbin, A. (1999) Role of etheno DNA adducts in carcinogenesis induced by vinyl chloride in rats, *IARC Sci. Publ.*, 303−313.
75. Osheroff, N. E. (1998) DNA Topoisomerases, *Biochim. Biophys. Acta 1400*.
76. 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.
77. Berger, J. M., and Wang, J. C. (1996) Recent developments in DNA topoisomerase II structure and mechanism, *Curr. Opin. Struct. Biol. 6*, 84−96.
78. Watt, P. M., and Hickson, I. D. (1994) Structure and function of type II DNA topoisomerases, *Biochem. J. 303*, 681−695.
79. Bromberg, K. D., Hendricks, C., Burgin, A. B., and Osheroff, N. (2002) Human topoisomerase IIα possesses an intrinsic nucleic acid specificity for DNA ligation. Use of 5′ covalently activated oligonucleotide substrates to study enzyme mechanism, *J. Biol. Chem. 277*, 31201−31206.
80. Bromberg, K. D., Velez-Cruz, R., Burgin, A. B., and Osheroff, N. (2004) DNA ligation catalyzed by human topoisomerase IIalpha, *Biochemistry 43*, 13416−13423.
81. Cullinan, D., Johnson, F., Grollman, A. P., Eisenberg, M., and de los Santos, C. (1997) Solution structure of a DNA duplex containing the exocyclic lesion 3,N^4-etheno-2′-deoxycytidine opposite 2′-deoxyguanosine, *Biochemistry 36*, 11933−11943.
82. Singer, B., Antoccia, A., Basu, A. K., Dosanjh, M. K., Fraenkel-Conrat, H., Gallagher, P. E., Kusmierek, J. T., Qiu, Z. H., and Rydberg, B. (1992) Both purified human 1,N^6-ethenoadenine-binding protein and purified human 3-methyladenine-DNA glycosylase act on 1,N^6-ethenoadenine and 3-methyladenine, *Proc. Natl. Acad. Sci. U.S.A. 89*, 9386−9390.
83. Rydberg, B., Qiu, Z. H., Dosanjh, M. K., and Singer, B. (1992) Partial purification of a human DNA glycosylase acting on the cyclic carcinogen adduct 1,N^6-ethenodeoxyadenosine, *Cancer Res. 52*, 1377−1379.
84. Wilson, S. H., and Kunkel, T. A. (2000) Passing the baton in base excision repair, *Nat. Struct. Biol. 7*, 176−178.
85. Felix, C. A. (2001) Leukemias related to treatment with DNA topoisomerase II inhibitors, *Med. Pediatr. Oncol. 36*, 525−535.
86. Felix, C. A. (1998) Secondary leukemias induced by topoisomerase-targeted drugs, *Biochim. Biophys. Acta 1400*, 233−255.
87. D'Arpa, P., and Liu, L. F. (1989) Topoisomerase-targeting antitumor drugs, *Biochim. Biophys. Acta 989*, 163−177.
88. D'Arpa, P., Beardmore, C., and Liu, L. F. (1990) Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons, *Cancer Res. 50*, 6919−6924.
89. Blair, I. A. (2001) Lipid hydroperoxide-mediated DNA damage, *Exp. Gerontol. 36*, 1473−1481.

BI0478289