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Etoposide Quinone Is a Redox-Dependent Topoisomerase II Poison

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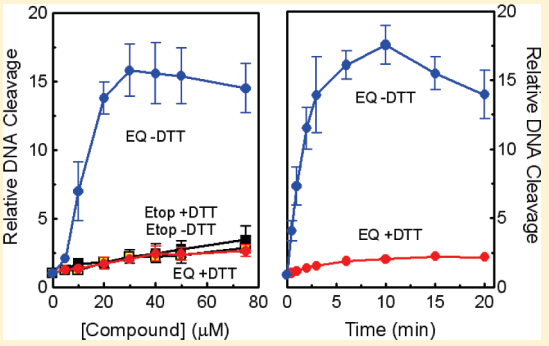
ABSTRACT: Etoposide is a topoisomerase II poison that is used to treat a variety of human cancers. Unfortunately, 2−3% of patients treated with etoposide develop treatment-related leukemias characterized by 11q23 chromosomal rearrangements. The molecular basis for etoposide-induced leukemogenesis is not understood but is associated with enzyme-mediated DNA cleavage. Etoposide is metabolized by CYP3A4 to etoposide catechol, which can be further oxidized to etoposide quinone. A CYP3A4 variant is associated with a lower risk of etoposide-related leukemias, suggesting that etoposide metabolites may be involved in leukemogenesis. Although etoposide acts at the enzyme−DNA interface, several quinones poison topoisomerase II via redox-dependent protein adduction. The effects of etoposide quinone on topoisomerase IIα-mediated DNA cleavage have been examined previously. Although findings suggest that the activity of the quinone is slightly greater than that of etoposide, these studies were carried out in the presence of significant levels of reducing agents (which should reduce etoposide quinone to the catechol). Therefore, we examined the ability of etoposide quinone to poison human topoisomerase IIα in the absence of reducing agents. Under these conditions, etoposide quinone was ∼5-fold more active than etoposide at inducing enzyme-mediated DNA cleavage. Consistent with other redox-dependent poisons, etoposide quinone inactivated topoisomerase IIα when incubated with the protein prior to DNA and lost activity in the presence of dithiothreitol. Unlike etoposide, the quinone metabolite did not require ATP for maximal activity and induced a high ratio of double-stranded DNA breaks. Our results support the hypothesis that etoposide quinone contributes to etoposide-related leukemogenesis.



Etoposide is a widely prescribed anticancer drug that is used as front-line treatment of a variety of human malignancies.[1−5] The compound is derived from podophyllotoxin, which is extracted from the May apple plant, and is currently in its fourth decade of clinical use.[1,2]

The primary cellular target of etoposide is topoisomerase II. Etoposide kills cells by inhibiting the ability of topoisomerase II to ligate DNA strand breaks that the enzyme generates as a requisite step in its double-stranded DNA passage reaction.[1−7] The drug stabilizes covalent topoisomerase II-cleaved DNA complexes (i.e., cleavage complexes) by interacting at the enzyme-cleaved DNA interface and converts this essential enzyme into a potent cellular toxin.[2−6] Because of the mechanism of action of etoposide, it is referred to as a topoisomerase II poison rather than a catalytic inhibitor.[2−5]

The collision of DNA tracking systems, such as the replication or transcription machinery, with the stabilized cleavage complexes creates permanent strand breaks in the genetic material.[1,2] The presence of these breaks induces DNA recombination/repair processes and has the potential to activate cell death pathways.[2−5] If cells survive drug treatment, they may carry chromosomal translocations or other aberrations.[8−10] To this point, while etoposide is a highly successful anticancer agent, ∼2−3% of patients treated with this drug go on to develop acute myeloid leukemia (AML).[11−14] The majority of these individuals carry balanced translocations that involve the mixed lineage leukemia (*MLL*) gene at chromosomal band 11q23.[8,9,14,15]

The molecular events that generate leukemic translocations in etoposide-treated cells are not well-defined. However, they are believed to be caused by the actions of the drug against topoisomerase II as opposed to an idiosyncratic effect.[8−14] Considerable evidence supports a mechanism by which the enzyme-linked strand breaks are processed to become translocation breakpoints.[10,15,16] However, it has been suggested that chromosomal breaks induced by abortive apoptotic DNA cleavage events (triggered by topoisomerase II-mediated DNA scission) also may play a role.[17,18]

In humans, etoposide can be metabolized by a number of pathways (Figure 1). Conversion of etoposide to a sulfate or a hydroxy acid inactivates the drug.[19,20] In contrast, E-ring modifications generated by the cytochrome P450 CYP3A4 convert one of the two methoxyl groups to a hydroxyl moiety, changing etoposide to a catechol metabolite that retains activity against human type II topoisomerases.[21−23] Etoposide catechol is present in the plasma of cancer patients that have been treated with regimens that include the parent drug.[13,21−27] The catechol can

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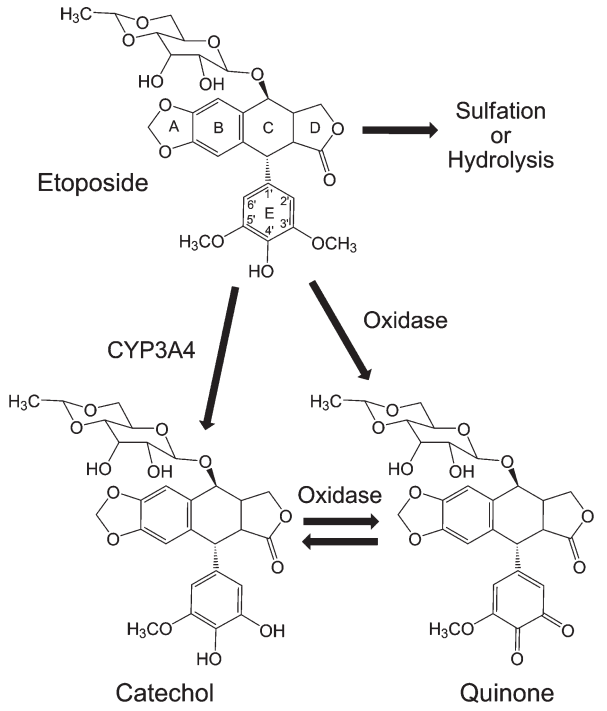

Figure 1. Metabolism of etoposide. The metabolism of etoposide can involve hydrolysis, sulfate modification, and demethylation (CYP3A4). While hydrolysis of etoposide yields hydroxy acid, the major metabolite, CYP3A4 metabolizes etoposide to the catechol. The parent compound or the catechol can be oxidized to produce the quinone. Myeloperoxidase may catalyze this oxidation in bone marrow progenitor cells. Adapted from ref 20.

be further oxidized to a quinone metabolite by the actions of cellular oxidases.[21−23,28−30] Etoposide quinone also can be generated from the phenoxyl radical that is produced by the one-electron oxidation of etoposide by peroxidases.[28,31,32] Like the catechol metabolite, etoposide quinone displays activity against topoisomerase II.[15,29,30]

A polymorphism in the 5′-promoter region of *CYP3A4* (i.e., *CYP3A4-V*), which is believed to decrease the cytochrome P450-mediated production of etoposide catechol, is associated with a lower risk of treatment-related AMLs that involve *MLL* gene translocations.[33] This finding suggests that etoposide metabolites may be involved in the leukemogenic process.

In cells, catechols can be oxidized to quinones by myeloperoxidase and other oxidases.[21−23,28,31,32,34] The high expression of myeloperoxidase in hematopoietic cells (∼3% by wt), together with the findings regarding CYP3A4, suggests that etoposide quinone may play a role in triggering *MLL*-associated AMLs.[15,31−33] In this regard, recent studies have shown that quinone-based compounds, such as 1,4-benzoquinone, NAPQI, and PCB quinone metabolites, are potent topoisomerase II poisons.[35−38] As compared to traditional (or interfacial) topoisomerase II poisons, such as etoposide, these quinones utilize an alternative redox-dependent mechanism that requires covalent attachment of the compound to the enzyme.[35,39,40] It is believed that protein adduction takes place on cysteine residues that are outside of the active site.[39,40]

Because of their potential roles in generating treatment-related leukemias, the effects of etoposide catechol and etoposide quinone on DNA cleavage mediated by human topoisomerase IIα have been examined.[15,29,30] Results indicate that both compounds display activity against the enzyme that is similar or slightly greater than that of etoposide. However, all of the earlier studies were carried out using buffers that contained significant levels of reducing agents such as dithiothreitol (DTT),[15,29,30] which should reduce etoposide quinone to the catechol.

Therefore, to more fully assess the ability of etoposide quinone to poison topoisomerase II, we characterized the activity of the compound against human topoisomerase IIα in buffers that carried minimal levels of reducing agent. In contrast to previous reports, etoposide quinone was found to be severalfold more efficacious than the parent compound at inducing enzyme-mediated DNA strand breaks. This enhanced activity was reduced to the level of etoposide when DTT was included in reactions. In addition, etoposide quinone demonstrated hallmark properties of redox-dependent topoisomerase II poisons. These findings suggest that the quinone metabolite of etoposide acts by an additional mechanism to stabilize topoisomerase II-DNA cleavage complexes and support a potential role for the compound in the generation of etoposide-induced AMLs.

EXPERIMENTAL PROCEDURES

Enzymes and Materials. Human topoisomerase IIα was expressed in *Saccharomyces cerevisiae* JEL1Δtop1 cells and purified as described previously.[41−43] The enzyme was stored at −80 °C as a 1.5 mg/mL (4 μM) stock in 50 mM Tris-HCl, pH 7.7, 0.1 mM EDTA, 750 mM KCl, 5% glycerol, and 40 μM DTT (carried from the enzyme preparation). Negatively supercoiled pBR322 DNA was prepared using a Plasmid Mega Kit (Qiagen) as described by the manufacturer. Etoposide and 1,4-benzoquinone were obtained from Sigma. Drugs were stored at 4 °C as 20 mM stock solutions in 100% DMSO.

Synthesis of Etoposide Quinone. Etoposide quinone was synthesized according to previously published procedures with slight modifications.[15,44] Briefly, etoposide (Sigma-Aldrich) (1 equiv) was dissolved in a 2:1 water:dioxane solution (20 mL/g) and treated with a 0.5 M aqueous solution of sodium metaperiodate (3 equiv) in the dark at 10 °C. After 40 min of stirring, the reaction solution was saturated with ammonium sulfate, extracted into dichloromethane, washed with brine, and dried with sodium sulfate. Removal of the solvent under reduced pressure yielded the crude compound. Purification was performed via flash chromatography using a silica column and a 0−10% methanol:dichloromethane gradient. Purity was determined as >99% by LCMS analysis at 220 and 254 nm in 72% yield.

Topoisomerase II-Mediated Cleavage of Plasmid DNA. Plasmid DNA cleavage reactions were performed using the procedure of Fortune and Osheroff.[45] Reaction mixtures contained 100 nM human topoisomerase IIα and 5 nM negatively supercoiled pBR322 DNA in 20 μL of 10 mM Tris-HCl, pH 7.9, 5 mM $MgCl_2$, 100 mM KCl, 0.1 mM EDTA, and 2.5% (v/v) glycerol. Final reaction mixtures contained ∼1 μM DTT, which represents the residual DTT carried along from the enzyme preparation. Unless stated otherwise, assays were started by the addition of enzyme, and DNA cleavage mixtures were incubated for 6 min at 37 °C. DNA cleavage reactions were carried out in the absence of compound or in the presence of 0−75 μM etoposide or etoposide quinone or 25 μM 1,4-benzoquinone, as indicated. In some cases, 250 μM DTT or 1 mM ATP was added to reaction mixtures. To examine the potential effects of

DNA adduction on topoisomerase IIα-mediated scission, 0.6 μg of pBR322 DNA was incubated with 30 μM etoposide quinone for 6 min at 37 °C in the absence of enzyme. Samples were then applied to a DNA Spin Column (Qiagen) and processed according to the manufacturer's protocol. DNA was eluted and added to DNA cleavage reactions (5 μL of the DNA eluate).

DNA cleavage complexes were trapped by the addition of 2 μL of 5% SDS followed by 2 μL of 250 mM Na_2EDTA, pH 8.0. Proteinase K was added (2 μL of a 0.8 mg/mL solution), and reaction mixtures were incubated for 30 min at 37 °C to digest topoisomerase IIα. Samples were mixed with 2 μL of agarose gel loading buffer (60% sucrose in 10 mM Tris−HCl, pH 7.9), heated for 2 min at 45 °C, and subjected to electrophoresis in 1% agarose gels in 40 mM Tris-acetate, pH 8.3, and 2 mM EDTA containing 0.5 μg/mL ethidium bromide. Double-stranded DNA cleavage was monitored by the conversion of negatively supercoiled plasmid DNA to linear molecules. DNA bands were visualized by UV light and were quantified using an Alpha Innotech digital imaging system (San Leandro, CA).

Topoisomerase II-Mediated Ligation of Plasmid DNA. DNA cleavage/ligation equilibria were established as described above for 6 min at 37 °C in the absence of compound or in the presence of 75 μM etoposide, 30 μM etoposide quinone, or 30 μM etoposide quinone with 250 μM DTT. Ligation was initiated by shifting samples from 37 to 0 °C. Reactions were stopped at time points ranging from 0 to 40 s by the addition of 2 μL of 5% SDS followed by 2 μL of 250 mM EDTA, pH 8.0. Samples were mixed with 2 μL of agarose gel loading buffer and processed and analyzed as described under plasmid DNA cleavage. Linear DNA cleavage product at time zero was set to 100%, and DNA ligation was monitored by the loss of linear DNA.

RESULTS AND DISCUSSION

Enhanced Activity of Etoposide Quinone in the Absence of Reducing Agents. Etoposide catechol and quinone (Figure 1) are etoposide metabolites that are generated by the actions of CYP3A4 and cellular oxidases/peroxidases.[21−23,28] The catechol metabolite has been found in the plasma of patients who have been treated with etoposide as part of a chemotherapeutic regimen.[13,21−27] Previous studies have examined the effects of these metabolites on human topoisomerase IIα and compared them to those of the parent compound.[15,29,30] In all cases, etoposide catechol and etoposide quinone enhanced enzyme-mediated DNA cleavage with activities that were similar or slightly greater than that of etoposide. However, these studies were carried out in the presence of relatively high levels of reducing agents, such as DTT, which were included in assay buffers or carried along as components of enzyme purification buffers.[15,29,30] Usually, DTT was present at concentrations that were in stoichiometric excess over the drugs. Thus, the bulk of etoposide quinone that was added to DNA cleavage assays was likely reduced to the catechol metabolite in these reaction mixtures.

Etoposide contains methoxyl substituents at the 3′- and 5′-positions of the E-ring. As determined by saturation transfer difference ^1H NMR spectroscopy, these methoxy groups contact topoisomerase II in the binary enzyme−drug complex.[46,47] While removal of both substituents reduces drug activity by ∼60%, removal of only one methoxyl group has little effect on activity.[46−48] Consequently, it is not surprising that etoposide catechol, which converts one of the methoxy groups to a hydroxyl moiety, can still interact in the active site of topoisomerase II and displays a drug activity that is similar to that of etoposide.

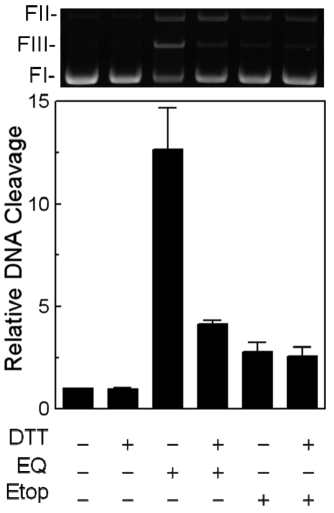

Figure 2. Etoposide quinone enhances topoisomerase II-mediated DNA cleavage in the absence of a reducing agent. Plasmid DNA cleavage was monitored using agarose gel electrophoresis (top) and quantified (bottom). The positions of supercoiled (form I, FI), nicked circular (form II, FII), and linear (form III, FIII) molecules are indicated at left. Reactions were performed with human topoisomerase IIα in the presence (+) or absence (−) of dithiothreitol (DTT) and no drug, 30 μM etoposide quinone (EQ), or 30 μM etoposide (Etop). The gel at the top is representative of three reactions. Error bars represent the standard deviation of three or more independent experiments.

In contrast to catechols, quinone-based compounds have the potential to poison topoisomerase II by a mechanism that is distinct from that of etoposide and other interfacial poisons.[35−38,49] These compounds, which require redox cycling, enhance topoisomerase II-mediated DNA cleavage by covalently adducting the protein at residues that are outside of the active site.[35−40] Redox-dependent topoisomerase II poisons also display other unique characteristics. Redox-dependent protein adduction is abrogated by exposure to reducing agents, which presumably converts the quinone back to the catechol.[38,49] In addition, while redox-dependent poisons stimulate enzyme-mediated DNA scission when added to the topoisomerase II−DNA complex, they inhibit DNA cleavage when incubated with the enzyme prior to the addition of DNA.[38,49]

Because of the potential role for etoposide metabolites in the induction of therapy-related leukemias, we characterized the activity of etoposide quinone against human topoisomerase IIα under conditions that maintained a high proportion of the quinone. By starting with a highly concentrated preparation of topoisomerase IIα and diluting the enzyme into buffers that were free of reducing agents, the residual DTT concentration in final reaction mixtures was held to less than 1 μM.

In contrast to previous reports, the activity of etoposide quinone against topoisomerase IIα was considerably higher than that of etoposide (Figure 2). At a drug concentration of 30 μM, etoposide quinone increased relative levels of enzyme-generated double-stranded DNA breaks >12-fold, as compared to ∼3-fold seen with etoposide. Furthermore, the activity of etoposide quinone dropped precipitously when 250 μM DTT was added to the reaction buffer, decreasing to a level that was comparable to that of etoposide.

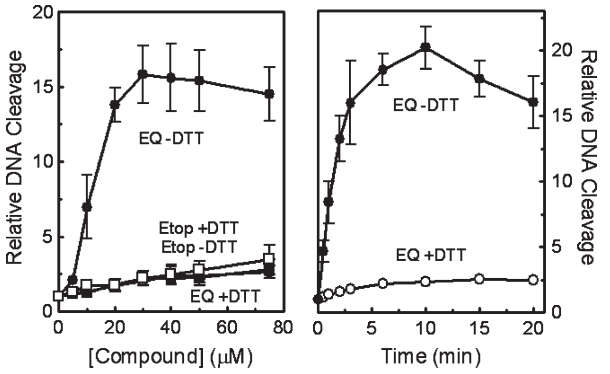

Figure 3. Etoposide quinone induces higher levels of DNA cleavage than etoposide in the absence of DTT. Left panel: plasmid DNA cleavage reactions were performed in the presence of increasing concentrations of etoposide or etoposide quinone with or without DTT. Closed circles represent etoposide quinone without DTT (EQ −DTT), open circles represent etoposide quinone with DTT (EQ +DTT), closed squares represent etoposide without DTT (Etop −DTT), and open squares represent etoposide with DTT (Etop +DTT). Right panel: a time course for DNA cleavage in the presence of 30 μM etoposide quinone with (EQ +DTT) or without (EQ −DTT) DTT is shown. Error bars represent the standard deviation of three independent experiments.

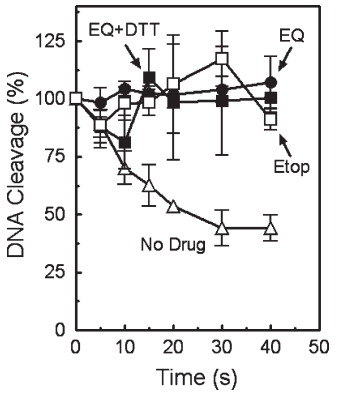

Figure 4. Etoposide quinone inhibits topoisomerase II-mediated DNA ligation. DNA cleavage reactions were initiated in the absence (open triangles, No Drug) or presence (closed circles, EQ) of 30 μM etoposide quinone, 30 μM etoposide quinone + DTT (closed squares, EQ + DTT), or 75 μM etoposide (open squares, Etop). The DNA cleavage/ligation equilibrium was established at 37 °C, and ligation was initiated by shifting samples to 0 °C. Reactions were stopped at time intervals from 0 to 40 s. The DNA cleavage observed at equilibrium for each reaction was set to 100% at time zero. Error bars represent the standard deviation of three independent experiments.

The concentration dependence of etoposide quinone was examined to further assess the ability of the metabolite to enhance DNA scission mediated by human topoisomerase IIα (Figure 3, left panel). At all concentrations examined, levels of DNA cleavage were severalfold higher in the presence of etoposide quinone than were observed with etoposide. Once again, the addition of excess DTT to reactions decreased the activity of the metabolite to the level seen with etoposide. In contrast, the presence of DTT had no effect on the activity of etoposide. Beyond its enhanced efficacy, the potency of etoposide quinone was higher than that of the parent compound. Maximal cleavage enhancement was observed at 30 μM etoposide quinone, whereas levels of DNA cleavage induced by etoposide were still rising at 75 μM drug. It is notable that in the presence of DTT the titration curve of etoposide quinone was similar to that of etoposide.

A time course for DNA cleavage mediated by topoisomerase IIα is shown in Figure 3 (right panel). At all time points examined, levels of scission induced by etoposide quinone were considerably higher in the absence of a reducing agent than in the presence of 250 μM DTT.

Etoposide as well as many redox-dependent topoisomerase II poisons increase levels of enzyme−DNA cleavage complexes by inhibiting the ability of the enzyme to ligate DNA. As seen in Figure 4, like the parent compound, etoposide quinone severely inhibited DNA ligation mediated by human topoisomerase IIα.

Etoposide Quinone Acts Primarily as a Redox-Dependent Topoisomerase II Poison. The inhibition of etoposide quinone by DTT strongly suggests that, in contrast to the parent compound, the metabolite is a redox-dependent topoisomerase II poison. However, it is possible that etoposide quinone simply acts as a more potent and efficacious interfacial topoisomerase II poison than either the parent compound or the catechol. To this point, the 4′-hydroxyl group on the E-ring is critical for the activity of etoposide.[46,47] Removal of this substituent significantly diminishes drug function but does not affect etoposide binding to topoisomerase II.[46] The addition of bulk to the 4′-position, such as by converting the hydroxyl to a methoxyl group, abrogates drug activity and also impairs etoposide binding to the enzyme.[47] However, it is not known how converting the 4′-hydroxyl group to a ketone in etoposide quinone impacts the ability of the drug to function as an interfacial topoisomerase II poison. While it could disrupt interactions of etoposide at the enzyme−DNA interface, it could also enhance them.

Therefore, several experiments were carried out to determine whether etoposide quinone acts primarily as a redox-dependent topoisomerase II poison or merely as a stronger interfacial poison. In the first experiment, the metabolite was incubated with topoisomerase IIα prior to the addition of DNA (Figure 5). As discussed above, redox-dependent topoisomerase II poisons inactivate the enzyme when the two are incubated in the absence of DNA.[38,49] In contrast, etoposide and other interfacial poisons do not impair topoisomerase II activity when coincubated in the absence of DNA.[38,49] As seen in Figure 5, incubation of 30 μM etoposide quinone with human topoisomerase IIα rapidly inactivated the enzyme. DNA cleavage activity was abolished within 30 s. In parallel experiments, incubation of the enzyme with etoposide or with a mixture of etoposide quinone and DTT had no effect on the cleavage activity of topoisomerase IIα.

The above data indicate that etoposide quinone can function as a redox-dependent topoisomerase II poison. However, they do not establish that the redox mechanism represents the primary mode by which the drug metabolite poisons the enzyme. Therefore, additional properties of drug activity were examined to further address this important point. In the second experiment, we assessed the ability of etoposide quinone to generate double-stranded vs single-stranded DNA breaks (Figure 6). Two molecules of etoposide are required to induce topoisomerase II-mediated double-stranded DNA breaks, one at each scissile bond.[6] Because the two drug molecules function independently, a high proportion of cleavage complexes established at subsaturating concentrations of etoposide contain only one cleaved DNA strand. Consequently, etoposide routinely induces double-stranded:single-stranded break ratios that approximate 0.5:1 (Figure 6).

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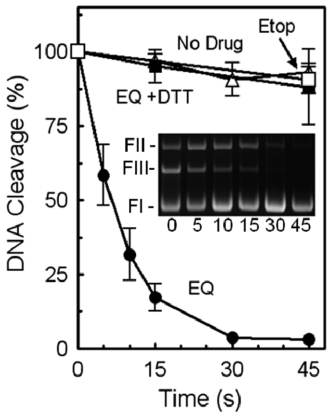

Figure 5. Etoposide quinone rapidly inactivates human topoisomerase IIα. Enzyme was incubated in the absence (open triangles, No Drug) or the presence of 30 μM etoposide quinone (closed circles, EQ), 30 μM etoposide quinone + DTT (closed squares, EQ + DTT), or 75 μM etoposide (open squares, Etop) for up to 45 s prior to a 6 min DNA cleavage reaction. Error bars represent the standard deviation of three independent experiments. The inset shows a representative agarose gel. The positions of supercoiled (form I, FI), nicked circular (form II, FII), and linear (form III, FIII) molecules are indicated at left.

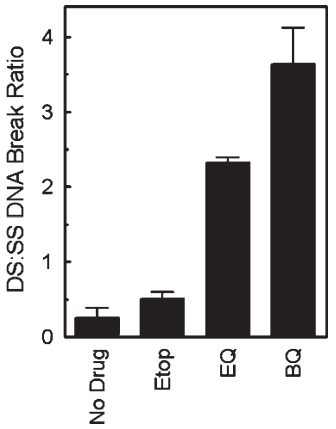

Figure 6. Etoposide quinone induces a high ratio of double-stranded to single-stranded DNA breaks. DNA strand breaks were monitored in reactions with no drug (No Drug), 30 μM etoposide (Etop), 30 μM etoposide quinone (EQ), or 25 μM 1,4-benzoquinone (BQ). Error bars represent the standard deviation of three independent experiments.

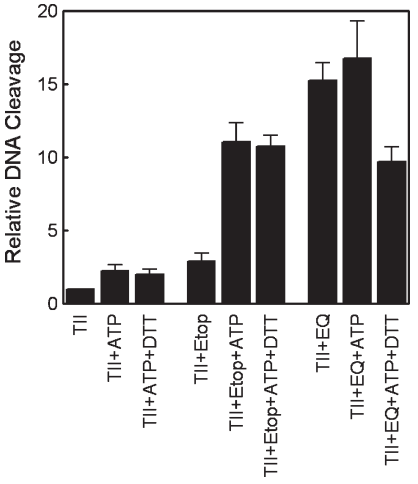

Figure 7. Etoposide quinone does not require ATP for optimal topoisomerase IIα-mediated DNA cleavage. Topoisomerase IIα (TII) DNA cleavage reactions were performed with no drug, 30 μM etoposide (Etop), or 30 μM etoposide quinone (EQ) in the presence and absence of ATP or DTT. Error bars represent the standard deviation of three independent experiments.

Conversely, since quinones (such as 1,4-benzoquinone) appear to function outside of the active site of topoisomerase II, protein adduction generates a double-stranded:single-stranded break ratio >1.[35] As seen in Figure 6, the proportion of topoisomerase IIα-mediated double-stranded DNA breaks induced by etoposide quinone (~2.3:1) more closely resembled that induced by 1,4-benzoquinone (~3.6:1) than etoposide.

Third, we examined the effects of ATP on the actions of etoposide quinone. It has long been known that etoposide requires ATP for maximal DNA cleavage activity.[50] As seen in Figure 7, etoposide-induced cleavage mediated by topoisomerase IIα rises ~3-fold when ATP is included in reaction mixtures. The presence of DTT did not affect this ATP-enhanced DNA cleavage. In contrast, levels of DNA cleavage induced by etoposide quinone in the presence of ATP were similar to those seen in the absence of a high-energy cofactor. It is notable that the addition of DTT to assay mixtures (which should convert etoposide quinone to the catechol) once again dropped levels of cleavage to those of etoposide.

Fourth, during the process of protein adduction, quinone-based compounds are reduced back to the hydroquinone (or catechol). Consequently, once a redox-dependent topoisomerase II poison has covalently attached to the enzyme, the presence of a reducing agent has no effect on the ability of the compound to stimulate DNA cleavage.[35] Therefore, an order of addition experiment was carried out (Figure 8). Topoisomerase IIα−DNA cleavage complexes were allowed to form in the presence of etoposide quinone and were further incubated for 6 min in the presence of H_2O or DTT. Once cleavage complexes were established, the addition of DTT did not affect levels of DNA scission. This is in contrast to results seen when DTT was added to reaction mixtures prior to the generation of cleavage complexes. As described above, reduction of etoposide quinone prior to DNA cleavage decreased levels of scission back to those seen with etoposide. These results are consistent with a topoisomerase II adduction mechanism for DNA cleavage enhancement by etoposide quinone.

Fifth, sites of quinone adduction to human topoisomerase IIα have been mapped to several cysteine residues.[40] Mutation of some of these residues to alanine, including Cys392, Cys405, or both (generating top2αC392A, top2αC405A, and top2α−C392A/C405A, respectively), affords partial protection against quinones.[40] In contrast, these mutant enzymes display wild-type activity in the absence of drugs or in the presence of interfacial poisons. To further assess the potential of etoposide quinone to function as a redox-dependent poison, the sensitivity of top2α−C392A/C405A to the compound was assessed (data not shown). Levels of DNA cleavage in the presence of etoposide quinone were ~20−25% lower than seen with wild-type topoisomerase IIα. While this decrease in cleavage is not as large as we observed with 1,4-benzoquinone (~50%), it is similar to what was seen with (−)-epigallocatechin gallate (~25%). (−)-Epigallocatechin gallate is a redox-dependent topoisomerase II poison found in

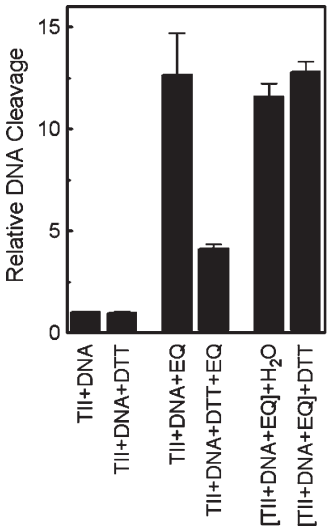

Figure 8. Etoposide quinone activity is not reversed by the addition of reducing agents after DNA cleavage complexes have been established. DNA cleavage reactions were carried out in the absence of etoposide quinone without (TII + DNA) or with DTT (TII + DNA + DTT) or in the presence of etoposide quinone without (TII + DNA + EQ) or with DTT (TII + DNA + EQ + DTT). Alternatively, after DNA cleavage complexes were form, reactions were further incubated in the presence of water ([TII + DNA + EQ] + H_2O) or DTT ([TII + DNA + EQ] + DTT) for an additional 6 min. Error bars represent the standard deviation of three independent experiments.

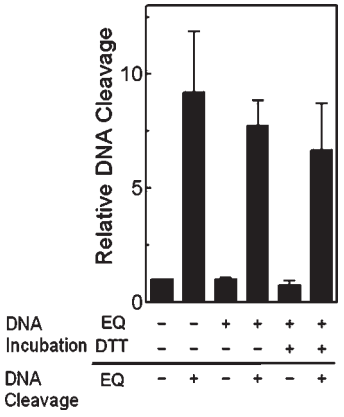

Figure 9. Etoposide quinone does not form DNA lesions that poison topoisomerase IIα. DNA was incubated (DNA Incubation) without (−) 30 μM etoposide quinone (EQ) or with (+) etoposide quinone in the absence or presence of DTT. DNA was purified from free drug and used in a DNA cleavage reaction (DNA Cleavage) with topoisomerase IIα. DNA cleavage reactions were performed in the absence (−) or presence (+) of 30 μM etoposide quinone. Error bars represent the standard deviation of three independent experiments.

green tea,[55] which, like etoposide quinone, is considerably larger than 1,4-benzoquinone. In contrast to the above results, no reduction in etoposide-induced DNA scission was observed with top2αC392A/C405A as compared to the wild-type enzyme.

The findings described above cannot rule out the possibility that a portion of etoposide quinone enhances topoisomerase IIα-mediated DNA cleavage by acting as an interfacial topoisomerase II poison. However, when taken together, the data strongly suggest that etoposide quinone poisons the human type II enzyme primarily by a redox-dependent mechanism.

Etoposide Quinone Does Not Poison Topoisomerase IIα by Adducting DNA. In addition to modifying proteins, quinones also can form covalent nucleic acid adducts, especially with the N7 of guanine residues.[51] The generation of quinone−DNA lesions could also enhance topoisomerase II-mediated DNA cleavage. Indeed, a number of studies have demonstrated that the presence of alkylated DNA lesions between the scissile bonds often increases levels of DNA scission.[52,53] Thus, it is possible that etoposide quinone could be stimulating topoisomerase IIα-mediated DNA cleavage by a mechanism that involves DNA, rather than protein, adduction.[15,54] To test this possibility, plasmid DNA was incubated with 30 μM etoposide quinone for 6 min at 37 °C and then purified from the quinone prior to DNA cleavage assays. As seen in Figure 9, this incubation had no effect on the DNA cleavage activity of topoisomerase IIα. Whether etoposide quinone was absent or present in final reaction mixtures, DNA cleavage levels were the same whether or not the plasmid had been exposed previously to the quinone. Thus, it is unlikely that etoposide quinone poisons topoisomerase II by a mechanism that involves a general adduction of DNA.

CONCLUSIONS

Etoposide quinone is a metabolite that is likely formed in patients who have been treated with the anticancer drug etoposide.[21−23,26,28] Although previous studies have suggested that that activity of etoposide quinone was similar to that of the parent compound, they were carried out in the presence of reducing agents that should convert the quinone back to a catechol.[15,29,30] The present study, which was carried out (essentially) in the absence of reducing agents, indicates that the activity of etoposide quinone against human topoisomerase IIα is actually severalfold higher than that of etoposide. Furthermore, in contrast to the parent compound, etoposide quinone appears to induce DNA cleavage by acting as a redox-dependent poison of the type II enzyme.

Unfortunately, ∼2−3% of cancer patients that are treated with etoposide eventually develop specific treatment-related AMLs.[11−14] The incidence of these AMLs appears to be lower in individuals who are deficient in CYP3A4, the enzyme that converts etoposide to the catechol.[33] Along with the results described above, this finding leads to the intriguing hypothesis that the oxidation of either the parent compound or the catechol to etoposide quinone by myeloperoxidases in hematopoietic cells generates a highly active redox-dependent topoisomerase II poison that contributes to the initiation of leukemogenic chromosomal rearrangements.

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