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Etoposide Catechol Is an Oxidizable Topoisomerase II Poison

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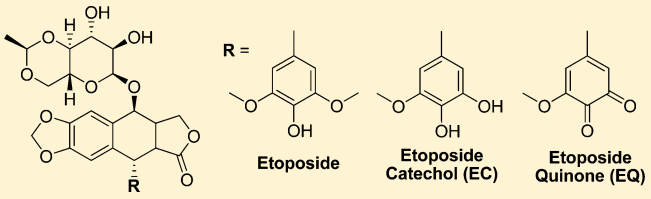
Supporting Information

ABSTRACT: Topoisomerase II regulates DNA topology by generating transient double-stranded breaks. The anticancer drug etoposide targets topoisomerase II and is associated with the formation of secondary leukemias in patients. The quinone and catechol metabolites of etoposide may contribute to strand breaks that trigger leukemic translocations. To further analyze the characteristics of etoposide metabolites, we extend our previous analysis of etoposide quinone to the catechol. We demonstrate that the catechol is ∼2−3-fold more potent than etoposide and under oxidative reaction conditions induces high levels of double-stranded DNA cleavage. These results support a role for etoposide catechol in contributing to therapy-induced DNA damage.



DNA topoisomerases are essential enzymes that are responsible for regulating the topology of DNA during transcription, replication, and mitosis.[1] The catalytic cycle of topoisomerase II involves the formation of a transient double-strand break with the 5′ ends of DNA covalently bound to active site tyrosines of the enzyme.[1,2] The covalent intermediate in this cycle is potentially genotoxic, and a number of widely used anticancer and antibacterial agents have been designed to take advantage of this cellular threat.[2] Most clinically relevant anticancer drugs that act on topoisomerase II do so by interacting noncovalently at the protein−DNA interface and inhibiting the ability of the enzyme to ligate the cleaved DNA. This turns the covalent enzyme-cleaved DNA intermediate into a stabilized single- or double-strand break. The anticancer agents etoposide and doxorubicin act in this interfacial manner and are categorized among a class of compounds known as topoisomerase II poisons.[2]

Aside from the interfacial topoisomerase II poisons, another class of compounds, formerly referred to as redox-dependent poisons, alter topoisomerase II activity using an adduction mechanism. Compounds in this class are now called covalent poisons.[3] Some compounds within this class, including quinones, have been shown to react with and form adducts with sulfhydryl groups on cysteine residues of topoisomerase II.[4−6] Covalent poisons induce high levels of double-stranded DNA cleavage when added to the enzyme−DNA complex[7] but display the unique feature of abrogating DNA cleavage if incubated with the enzyme prior to the nucleic acid substrate.[4,5,7] This effect, referred to as inactivation, is seen with a number of covalent poisons and is likely the result of covalent adduction.[6]

Etoposide is an interfacial topoisomerase II poison that is used to treat a wide range of solid and hematologic malignancies.[8] Unfortunately, 2−3% of patients treated with etoposide develop secondary leukemias that frequently involve specific translocations at chromosome band 11q23.[9,10] Etoposide may contribute to translocations via the interfacial poisoning mechanism; however, there are metabolites of etoposide formed *in vivo* that also may contribute to the promotion of stabilized double-strand breaks potentially using both interfacial and covalent poisoning mechanisms. Etoposide is metabolized by CYP3A4 in the liver to a catechol metabolite,[11,12] which may be further oxidized to a quinone metabolite.[13,14] Both of these metabolites have activity against topoisomerase II.[7,14−16] Studies on these metabolites were originally performed more than a decade ago and showed that the compounds induced cleavage levels similar to or slightly higher than those relative to the etoposide.[14−16] However, these early studies involved high concentrations of reducing agents (DTT) in the reaction buffers and/or the enzyme storage buffer.[14−16] Consequently, compounds were maintained in a reduced state and were not assessed for the ability to act as covalent poisons.

Previously, we synthesized and characterized etoposide quinone in the absence of reducing agents.[7] Our results showed that etoposide quinone acts in a manner similar to that of other covalent poisons of topoisomerase II and induces ∼5-fold higher cleavage levels than etoposide. The quinone stabilizes an ∼4-fold higher ratio of double-stranded to single-stranded breaks when compared to that of etoposide. Further, the quinone can inactivate DNA cleavage in a manner similar to that of other covalent poisons when incubated with topoisomerase II prior to DNA. The presence of reducing

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agents prevents the quinone from acting as a covalent poison and results in an interfacial poisoning mechanism with cleavage levels slightly higher than those seen with etoposide.

In the present study, we synthesized etoposide catechol and characterized its activity against human topoisomerase IIα in order to determine whether it can act as a covalent topoisomerase II poison. The catechol inhibits plasmid DNA relaxation, as seen in previous studies[14−16] (Supporting Information, Figure S1), likely due to its ability to inhibit ligation (Supporting Information, Figure S2). Furthermore, in the absence of reducing agents, etoposide catechol induces 2-fold higher levels of DNA cleavage in the 5−50 μM range as compared to etoposide (Figure 1). At catechol concentrations

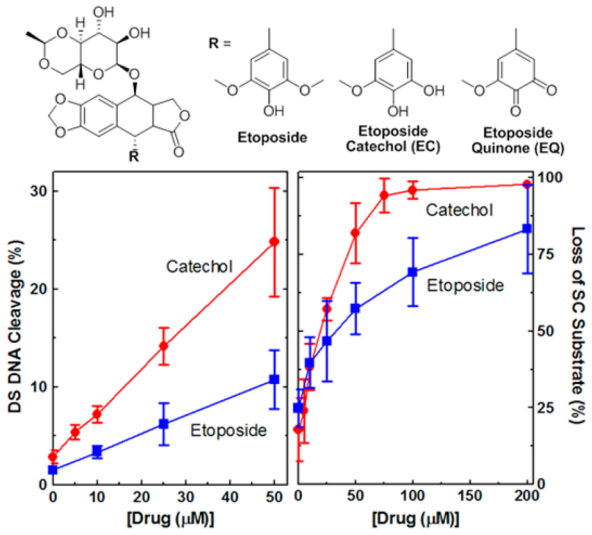

Figure 1. Topoisomerase IIα-mediated plasmid DNA cleavage levels are higher in the presence of etoposide catechol than etoposide. Upper panel: structure of etoposide and the catechol and quinone metabolites. Left panel shows the percent of DS DNA cleavage in the presence of increasing concentrations of either etoposide (blue) or the catechol (red). Due to plasmid fragmentation at catechol concentrations above 50 μM, DNA cleavage was also monitored by measuring the loss of supercoiled substrate in the presence of etoposide (blue) or catechol (red) (right panel). Error bars represent the standard deviation of three or more independent experiments.

above 50 μM, plasmids are fragmented resulting from the stabilization of two or more topoisomerase II−DNA cleavage complexes on each plasmid (Supporting Information, Figure S3). Similar results are seen under these conditions with the quinone (data not shown).

To determine how the catechol stabilizes high levels of DNA cleavage, we examined whether the compound can be oxidized to the quinone form. To oxidize the catechol, we incubated the drug with the oxidizing agent potassium ferric cyanide ($K_3F(CN)_6$), similar to that in a recent study with oxidized curcumin metabolites.[17] Levels of topoisomerase IIα-mediated DNA cleavage in the presence of $K_3F(CN)_6$ increase 1.5−2-fold at 25 μM oxidized catechol when compared to reactions without oxidizing agent or those with DTT (Figure 2). In the presence of the reducing agent DTT, cleavage levels with the catechol are still 2-fold higher than those seen with etoposide (Figure 2 and Supporting Information, Figure S4). In contrast, the addition of $K_3F(CN)_6$ to etoposide has only a mild effect on DNA cleavage, and the oxidizing agent alone does not significantly induce DNA cleavage (Figure 2). On the basis of

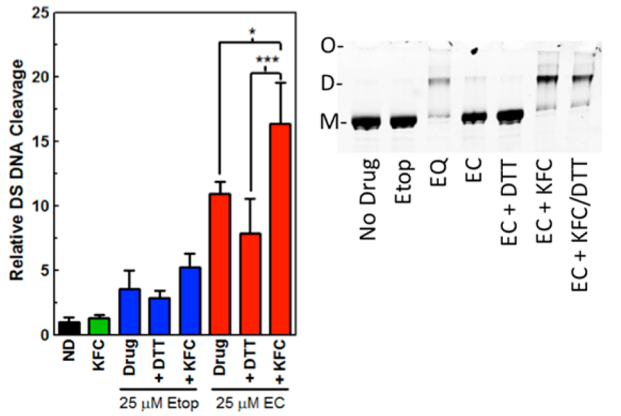

Figure 2. Etoposide catechol can be oxidized to the quinone. Left panel: topoisomerase IIα-mediated DS DNA cleavage levels are shown for reactions with no drug (black), $K_3F(CN)_6$ alone (KFC, green), 25 μM etoposide (blue), or 25 μM etoposide catechol (red). Reactions with etoposide or catechol were performed with drug alone (Drug), with the addition of DTT (+DTT), or with the addition of $K_3F(CN)_6$ (+KFC). Statistically significant changes in mean are denoted by asterisks (* is $p < 0.05$; *** is $p < 0.001$). Error bars represent the standard deviation of three or more independent experiments. Right panel: SDS−PAGE gel image displaying covalent cross-linking of enzyme monomers (M) to form dimers (D) in the presence of 50 μM Etop, EQ, or EC. EC was reacted with DTT (+DTT) or KFC (+KFC) or reacted with KFC followed later by DTT to test reversibility of the adduction (+KFC/DTT). Origin (O) is denoted. The image is representative three independent experiments.

mass spectrometry, the oxidation of the catechol by $K_3F(CN)_6$ generates the quinone (data not shown). In addition, the oxidation product covalently cross-links the two protomers of the dimeric enzyme, which cannot be reversed by the addition of DTT after the reaction (Figure 2). The location(s) and mechanism of the adduction by the quinone are still under investigation.

Since double-stranded/single-stranded (DS:SS) DNA break ratios with etoposide quinone and benzoquinone are >4-fold higher than that of etoposide,[7] we compared the DS:SS DNA break ratios for etoposide catechol (Figure 3). As seen in Figure 3, catechol in the presence of $K_3F(CN)_6$ results in ∼4-fold higher DS:SS DNA break ratios when compared to that of etoposide or the catechol in the absence of an oxidizing agent.

Additionally, covalent poisons abrogate topoisomerase II-mediated DNA cleavage when incubated with the enzyme prior to the addition of DNA.[4,5,7] Therefore, we examined whether oxidation enabled this compound to inactivate DNA cleavage by topoisomerase IIα. As seen in Supporting Information, Figure S5, only oxidized etoposide catechol inactivates topoisomerase IIα in the context of the 45 s time scale in the experiment when incubated with the enzyme prior to the addition of plasmid substrate. Further, $K_3F(CN)_6$ does not inactivate the enzyme on its own (Supporting Information, Figure S6). These results with oxidized catechol are similar to those seen with etoposide quinone and other covalent poisons.[4,5,7]

Our results show that etoposide catechol can inhibit ligation, which is consistent with previous work of the metabolite under reducing conditions.[14−16] In contrast to previous reports, we also demonstrate that the catechol is more potent in the absence of reducing agents and can be oxidized to the quinone form that stabilizes high levels of double-stranded DNA

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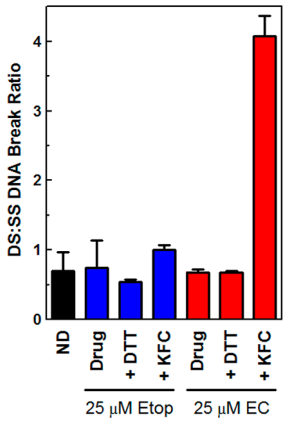

Figure 3. Double-strand to single-strand DNA break ratios were compared for cleavage reactions without the drug (ND), with 25 μM etoposide (blue), or with 25 μM etoposide catechol. Reactions with etoposide or catechol were performed with the drug alone (Drug), with the addition of DTT (+DTT), or with the addition of $K_3F(CN)_6$ (+KFC). Error bars represent the standard deviation of three or more independent experiments.

cleavage and covalently cross-links the enzyme protomers. Therefore, we propose that the mechanism by which the catechol metabolite poisons topoisomerase II is oxidation-dependent acting as (1) an interfacial poison under reducing conditions or (2) a covalent poison, in the quinone form, under oxidizing conditions.

Since the P450-generated catechol metabolite of etoposide has been observed in patient sera at nanomolar levels, it is possible for these compounds to impact patient outcomes.[18−20] Due to the covalent cross-linking mechanism of the quinone, we hypothesize that etoposide metabolites are present at sufficient levels to enable them to cause permanent strand breaks within the hematopoietic precursor cells without overwhelming these cells with fatal damage. When cells attempt to remove the cross-linked enzyme protomers and repair the DNA strand breaks, leukemogenic translocations can take place. While this model for the involvement of etoposide metabolites in contributing to secondary leukemias has support, more work will be needed to establish the contribution of the metabolites to damage in cells.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental methods including catechol synthesis and figures displaying DNA relaxation, ligation, cleavage, and enzyme inactivation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CYP3A4, cytochrome P450 3A4; $K_3F(CN)_6$, KFC, potassium ferricyanite; DTT, dithiothreitol; DS, double-stranded; Etop, etoposide; EC, etoposide catechol; EQ, etoposide quinone

■ REFERENCES

(1) Wang, J. C. (2002) *Nat. Rev. Mol. Cell Biol. 3*, 430−440.
(2) Deweese, J. E., and Osheroff, N. (2009) *Nucleic Acids Res. 37*, 738−749.
(3) Ketron, A. C., and Osheroff, N. (2013) *Phytochem. Rev.*, DOI: 10.1007/s11101-013-9291-7.
(4) Lindsey, R. H., Jr., Bromberg, K. D., Felix, C. A., and Osheroff, N. (2004) *Biochemistry 43*, 7563−7574.
(5) Lindsey, R. H., Jr., Bender, R. P., and Osheroff, N. (2005) *Chem. Res. Toxicol. 18*, 761−770.
(6) Bender, R. P., Ham, A. J., and Osheroff, N. (2007) *Biochemistry 46*, 2856−2864.
(7) Jacob, D. A., Mercer, S. L., Osheroff, N., and Deweese, J. E. (2011) *Biochemistry 50*, 5660−5667.
(8) Hande, K. R. (1998) *Eur. J. Cancer 34*, 1514−1521.
(9) DeVore, R., Whitlock, J., Hainsworth, T., and Johnson, D. (1989) *Ann. Intern. Med. 110*, 740−742.
(10) Felix, C. A. (2001) *Med. Pediatr. Oncol. 36*, 525−535.
(11) van Maanen, J. M., de Vries, J., Pappie, D., van den Akker, E., Lafleur, V. M., Retel, J., van der Greef, J., and Pinedo, H. M. (1987) *Cancer Res. 47*, 4658−4662.
(12) Relling, M. V., Nemec, J., Schuetz, E. G., Schuetz, J. D., Gonzalez, F. J., and Korzekwa, K. R. (1994) *Mol. Pharmacol. 45*, 352−358.
(13) van Maanen, J. M., Verkerk, U. H., Broersen, J., Lafleur, M. V., De Vries, J., Retel, J., and Pinedo, H. M. (1988) *Free Radical Res. Commun. 4*, 371−384.
(14) Lovett, B. D., Strumberg, D., Blair, I. A., Pang, S., Burden, D. A., Megonigal, M. D., Rappaport, E. F., Rebbeck, T. R., Osheroff, N., Pommier, Y. G., and Felix, C. A. (2001) *Biochemistry 40*, 1159−1170.
(15) Gantchev, T. G., and Hunting, D. J. (1997) *Biochem. Biophys. Res. Commun. 237*, 24−27.
(16) Gantchev, T. G., and Hunting, D. J. (1998) *Mol. Pharmacol. 53*, 422−428.
(17) Ketron, A. C., Gordon, O. N., Schneider, C., and Osheroff, N. (2013) *Biochemistry 52*, 221−227.
(18) Stremetzne, S., Jaehde, U., Kasper, R., Beyer, J., Siegert, W., and Schunack, W. (1997) *Eur. J. Cancer 33*, 978−979.
(19) Cai, X., Woo, M. H., Edick, M. J., and Relling, M. V. (1999) *J. Chromatogr., B 728*, 241−250.
(20) Zheng, N., Felix, C. A., Pang, S., Boston, R., Moate, P., Scavuzzo, J., and Blair, I. A. (2004) *Clin. Cancer Res. 10*, 2977−2985.

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