Anti-Cancer Drugs 1999, **10**, pp. 397–404

Preclinical report

A unique paclitaxel-mediated modulation of the catalytic activity of topoisomerase IIα

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Paclitaxel (Taxol) is known to act by polymerizing and stabilizing microtubules. In spite of a known target, the existence of additional targets is suggested by a poor understanding of the mechanism(s) underlying *eventual* cell death by paclitaxel and by the drug's high efficacy, as compared to other spindle poisons. Based on the enhanced sensitivity of a mutant DNA double-strand break repair-deficient Chinese hamster ovary cell line to paclitaxel as well as to various topoisomerase (Topo) II poisons, it was hypothesized that paclitaxel, in addition to having an effect on microtubules, may also alter the activity of Topo II. This study demonstrates the unique, *in vitro* effects of paclitaxel on Topo II activity as investigated by monitoring the decatenation of kinetoplast DNA and relaxation of supercoiled plasmid DNA by Topo II. Unlike classical anti-topoisomerase drugs, low concentrations of paclitaxel (0.02–500 nM) stimulated Topo II catalytic activity, while higher concentrations over 5 μM inhibited the activity of Topo II. Furthermore, these effects of paclitaxel appear to be mediated through a direct interaction of paclitaxel with Topo II rather than an interaction with DNA or DNA–Topo II complexes. Collectively, the evidence presented suggests the existence of an atypical interaction between Topo II and paclitaxel that may disrupt the normal functioning of the enzyme. [© 1999 Lippincott Williams & Wilkins.]

Key words: Distamycin, kDNA decatenation assay, paclitaxel, pBR322 relaxation assay, Taxol, topoisomerase II.

Introduction

Paclitaxel (Taxol) is categorized as a mitotic spindle poison.[1] Its excellent antitumor activity against various types of cancers, including ovarian and breast cancers, has lead to a large body of research, investigating the mechanistic aspects of paclitaxel's action in cancer cells. Cell cycle arrest following microtubule stabilization by paclitaxel leads to cell death by mechanisms that are still poorly understood. Also, in spite of a known target, paclitaxel's cytotoxic efficacy surpasses its microtubule-mediated effects (as compared to other spindle poisons) and this has lead to speculations about the existence of additional targets of paclitaxel in cancer cells.

Topoisomerase (Topo) II, an essential nuclear enzyme, controls DNA topology by causing transient DNA double-strand breaks (DSBs) in the DNA double helix and rejoining them after the passage of a second intact DNA molecule through the break.[2] *In vitro*, this Topo II-mediated DNA passage reaction can be studied by a number of catalytic assays, including catenation/decatenation, relaxation and knotting/unknotting of DNA (reviewed by Boege[3]). Topo II plays an important role during mitosis, DNA replication and recombination. It exists as two isoforms (Topo IIα and β), which are regulated by the cell independently. Some of the most efficacious anticancer drugs target this enzyme, especially Topo IIα, to cause their cytotoxicity (reviewed in Pommier *et al.*[2]).

The study reported here was based on the previously observed differential sensitivity of a pair of mutant Chinese hamster ovary (CHO) cell lines, xrs-6 and BR1.[4] xrs-6 cells are a mutant strain of CHO-K1 cells, that are deficient in DNA DSB repair.[5] The BR1 cell line, isolated on the basis of its resistance to bis-chloroethylnitrosourea, is DNA DSB repair competent.[6] xrs-6 cells have been previously shown to be hypersensitive to *m*-AMSA and etoposide.[7,8] Using a

Both authors contributed equally to this work. This work was supported in part by NIH grant CA63440, Sigma Xi Grants-in-Aid of Research and the School of Pharmacy, Northeast Louisiana University.

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modified MTT assay, Swaffar *et al.* demonstrated an enhanced sensitivity of xrs-6 cells to all the clinically available Topo II poisons as compared to BR1 cells but not to other classes of anticancer drugs[4] (except bleomycin which causes DSBs by a free radical-mediated mechanism[9]). We now report that xrs-6 cells are also several-fold more sensitive to paclitaxel than BR1 cells. Based on this difference, we hypothesized that, in addition to targeting microtubules, paclitaxel may interact, directly or indirectly, with Topo II. This study presents evidence supporting our hypothesis that paclitaxel, in addition to targeting microtubules, also causes a unique concentration-dependent alteration of Topo II's catalytic activity. The high efficacy of paclitaxel, compared to other mitotic spindle poisons, against cancer cells may be due to a combined effect on microtubules and Topo II.

Materials and methods

Cell cultures

The CHO cell lines, xrs-6 and BR1, were maintained as monolayer cultures in α-minimal essential medium (α-MEM; Life Technologies, Grand Island, NY) as previously described.[4] Cells were confirmed to be free of mycoplasma contamination using the Gen Probe hybridization kit (Gen-Probe, San Diego, CA).

Drugs and chemicals

Paclitaxel and *m*-AMSA were kind gifts from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI (Bethesda, MD). Some paclitaxel was also obtained from ICN Chemicals (Costa Mesa, CA). Drugs were dissolved in DMSO immediately before use. Distamycin, Hoechst 33258 and calf thymus DNA were purchased from Sigma (St Louis, MO). Purified human Topo IIα, kDNA, and linear and decatenated kDNA markers were obtained from TopoGen (Columbus, OH). Supercoiled plasmid DNA, pBR322, was supplied by New England Biolabs (Beverly, MA). All other reagents were of analytical grade.

Microtiter tetrazolium assay

The cellular response of xrs-6 and BR1 cells to paclitaxel was evaluated by modifications to the MTT assay as previously reported.[4] The ratio of the IC_{50} of paclitaxel against BR1 cells to its IC_{50} against the xrs-6 cells was determined.

Decatenation assay

To test the possible effects of paclitaxel on the catalytic activity of Topo II, a cell-free *in vitro* system was used in which the decatenation of kDNA by Topo II was monitored in the presence of paclitaxel. Reaction volumes of 25 μl consisted of 0.2 μg kDNA, 1 μl of appropriate drug concentration or solvent (DMSO), 1 μl of human Topo IIα in reaction buffer containing 50 mM Tris-HCl, pH 8.0, 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol and 30 μg BSA/ml. Samples were incubated at 37°C for 30 min and reactions stopped by the addition of 5 μl of buffer containing 5% sarkosyl, 0.0025% bromophenol blue and 25% glycerol. Samples were loaded on a 1% agarose gel and run overnight at 20 V in 1 × Tris-acetate-EDTA (TAE) buffer containing 0.5 μg/ml ethidium bromide. Decatenated kDNA was visualized under UV light. Digitized images were captured and analyzed using the FotoAnalyst II system (Fotodyne, Hartland, WI) and NIH-Image software. Results shown are an average of three to six independent experiments.

Relaxation assay

The catalytic activity of purified Topo II was also evaluated by monitoring the relaxation of supercoiled plasmid DNA, pBR322, in the presence of paclitaxel. The reaction volumes were similar to the decatenation assay except that pBR322 DNA was used as the Topo II substrate and incubations were for 20 min. The relaxed DNA was separated from supercoiled DNA by overnight electrophoresis in 1 × TAE (without ethidium bromide) at 20 V. For titration studies, increasing concentrations of Topo IIα or DNA were added to the reaction mixtures containing an inhibitory concentration of paclitaxel (50 μM). Gels were processed as described above except that they were stained with ethidium bromide after the run followed by destaining in deionized water.

Paclitaxel–DNA binding studies

Binding of paclitaxel to DNA, if any, was monitored by a fluorescence-quenching assay. Purified calf thymus DNA was used as the DNA substrate and Hoechst 33258, a DNA binding dye, was used as the fluorophore. Fluorescence emission spectra were obtained at 455 nm on a PTI QuantaMaster spectrofluorometer (PTI, Brunswick, NJ) with excitation wavelength set at 365 nm. All data points were acquired at 25°C. Assay mixtures were comprised of

10 μg DNA and 0–100 μM paclitaxel or *m*-AMSA, a DNA intercalator used as a positive control, in 2 ml of 1 × Tris–sodium chloride–EDTA (TNE) buffer, pH 7.4 (10 mM Tris, 1 mM Na_2EDTA and 100 mM NaCl) containing 1 μg/ml Hoechst 33258 dye. Samples were incubated for 10 min at 37°C prior to fluorescence measurements. All reagents and water used in the experiments were ultra pure grade to reduce any non-specific fluorescence.

Results

Differential sensitivity of xrs-6 and BR1 cells to paclitaxel

A screen for agents that cause DNA DSBs has been validated by Swaffar *et al.*[4] This work has lead to the discovery of the makaluvamines, a new class of Topo II inhibitors isolated from a marine sponge.[10] This screen uses a pair of mutant CHO cells, BR1 and xrs-6, and examines any differential cytotoxicity of agents towards them. xrs-6 cells are deficient in the repair of DNA DSBs, while BR1 cells are proficient in this repair. Based on a deficiency in V(D)J recombinase activity, the xrs-6 cells probably attempt to repair breaks in their double-strand DNA, but only illegitimate joins are produced.[11] Accordingly, it would take less DSBs to kill these cells and thus they are much more sensitive to killing by agents that induce such damage. Since Topo II inhibitors induce DNA DSBs, xrs-6 cells are hypersensitive to them.

The IC_{50} values of paclitaxel for xrs-6 and BR1 cells (approximately 0.95 and 6 μM, respectively) indicated a more than 6-fold difference in the cell's sensitivities to paclitaxel. Using the same assay, a parallel differential response for other Topo II poisons has been reported earlier.[4] For example, etoposide, a classical Topo II poison, was found to be 7-fold more cytotoxic to xrs-6 than BR1 cells in that study. On the other hand, for non-Topo II interacting drugs such as mitomycin C or camptothecin, both cell lines were found to be equally sensitive to the cytotoxic agent used.[4] The large difference in sensitivity of xrs-6 and BR1 cells to paclitaxel suggests that it may induce DSBs and it is reasonable to infer that these DSBs may be mediated by Topo II interactions.

Dual effects of paclitaxel on the catalytic activity of Topo II

In order to determine if the observed differential sensitivity of xrs-6 cells to paclitaxel is a result of our hypothesized disruption of the normal functioning of Topo II by paclitaxel, we investigated the effects of paclitaxel on Topo II in an *in vitro* system using purified Topo IIα. Topo II activity was assayed using both a kinetoplast DNA (kDNA) and a pBR322 relaxation assay.

Decatenation assay. kDNA is a catenated network of DNA rings which is too large to enter the gel. Topo II, due to its ability to decatenate kDNA, generates monomeric DNA molecules which can be separated from the networks by horizontal gel electrophoresis. Following ethidium bromide staining these monomers can be detected under UV light. In the presence of increasing concentrations of a Topo II inhibitor such as etoposide, one expects to see a dose-dependent decrease in the decatenation of kDNA by Topo II (as evidenced by the progressive disappearance of monomer bands). Results of these experiments consistently displayed a dual pattern of paclitaxel effects on Topo II activity (Figure 1). A representative gel photograph of the results of a typical decatenation assay is shown in Figure 1(A). Figure 1(B) shows the densitometric quantitation of decatenated kDNA by Topo II in the presence of paclitaxel for three to six independent experiments. The absence of any decatenated kDNA in the first lane of Figure 1(A) confirms the purity of catenated kDNA substrate without any contaminating decatenated kDNA. Lane 13 shows the effect of solvent (DMSO) in the absence of any drug and was used to compare the effect of paclitaxel on Topo II. In the absence of Topo II, paclitaxel had no effect on catenated kDNA and did not cause any nicking of the DNA (data not shown). The dual nature of paclitaxel's effect on Topo IIα is clearly depicted in densitometric quantitation of the results from three to six independent experiments (Figure 1B). At lower concentrations (0.02–200 nM paclitaxel), a dose-dependent increase up to approximately 70% was seen in the amount of kDNA decatenated over the solvent control, while at higher concentrations (5–100 μM paclitaxel), decatenation of kDNA was gradually decreased to zero. The average of these experiments showed that approximately 0.05 μM paclitaxel caused a maximal stimulation of Topo II's catalytic activity, while 10 μM paclitaxel inhibited Topo II-mediated decatenation of kDNA by 50%.

Relaxation assay. Due to the unique nature of paclitaxel's effects on Topo II, confirming results were sought using a different assay for catalytic activity of the enzyme. Results of the relaxation assay, shown in Figure 2, consistently followed a pattern similar to the decatenation assay. Topo II, by virtue of its ability to

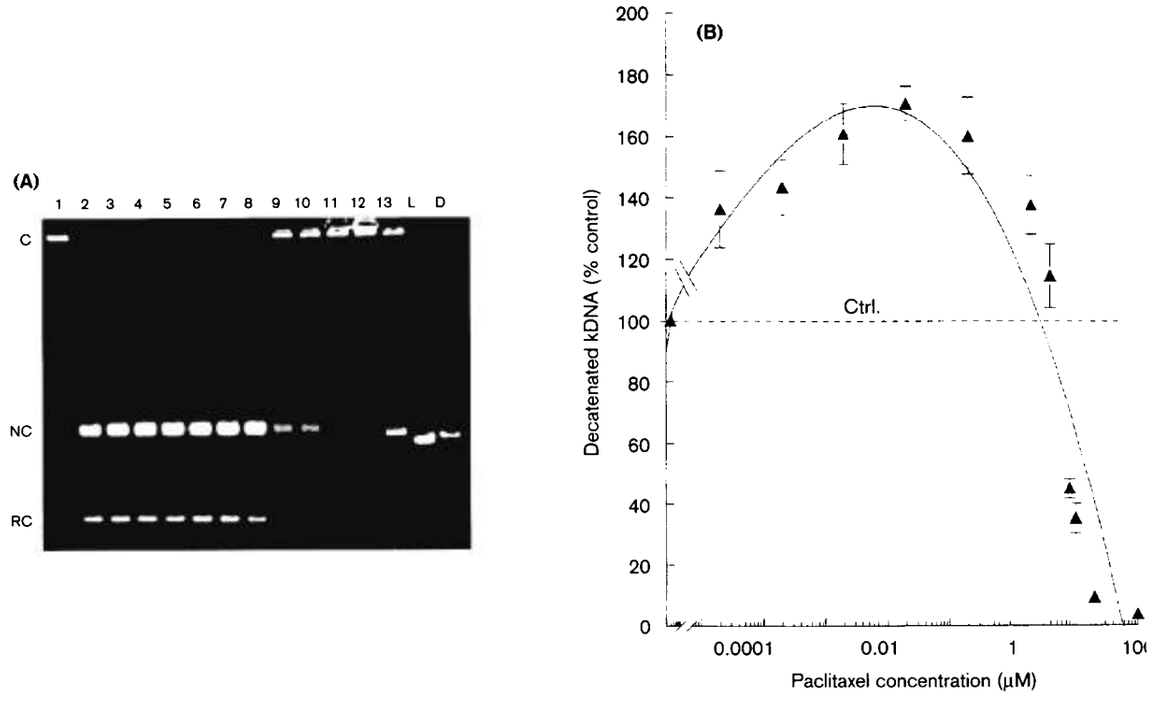

Figure 1. Dual effects of paclitaxel on Topo II-mediated decatenation of kDNA. (A) A representative gel photograph showing decatenation of 0.2 μg kDNA by purified Topo II in the presence of varying concentrations of paclitaxel as monitored by horizontal gel electrophoresis. Gel was run overnight in 1 × TAE containing 0.5 μg/ml ethidium bromide and processed as described in Materials and methods. Lane 1, kDNA substrate; lane 13, solvent (DMSO) control (only one of these controls is shown in this portion of the gel; typically two control lanes were run); lanes 2–12, 0.00002, 0.0002, 0.002, 0.02, 0.2, 2, 4, 8, 10, 20 and 100 μM paclitaxel. The last two lanes represent the marker kDNA to locate the position of decatenated kDNA bands in sample lanes. L, linear kDNA; D, decatenated kDNA; C, catenated networks; NC, nicked circular decatenated DNA; RC, relaxed circular decatenated DNA. (B) Densitometric analysis of digitized images of gels. Optical densities of decatenated kDNA bands in presence of varying concentrations of paclitaxel, measured as AUC, were determined using Image and are represented as percent solvent control. Points, the mean of three to six independent experiments; bars, SEM.

resolve topological DNA tension using double-strand passage mechanism, can relax supercoiled DNA (form I, FI) into relaxed forms of DNA, some of which co-migrate with nicked circular DNA (form II, FII) and thus can be separated from the supercoiled form.[12] A Topo II inhibitor suppresses the Topo II-mediated DNA relaxation leading to reappearance of the supercoiled DNA band. Since distamycin, a minor groove binder, has been shown to modulate the catalytic activity of Topo II in a dual fashion,[13] it was included as a positive control. Lane 1 in Figure 2 shows pBR322 DNA (in the absence of Topo II or drug) which consists of approximately 20% nicked and 80% supercoiled DNA. In the absence of drug, the amount of Topo II added converted more than 50% of supercoiled DNA (FI) into relaxed forms (Figure 2, lanes 2 and 9). Like the decatenation assay, Figure 2 clearly shows the dual pattern of paclitaxel's action on Topo II. Our positive control, distamycin, stimulated the relaxation of DNA (Figure 2, lanes 4 and 5) at low concentrations (0.5–1.0 μM), while higher concentrations (10 μM or more; Figure 2, lanes 7 and 8) inhibited it. In comparison, paclitaxel exhibited a parallel effect with a slightly different concentration profile. Stimulation of Topo II-mediated DNA relaxation, which was evident at 0.01 μM (Figure 2, lane 11), was found to be maximal at 0.5 μM (Figure 2, lane 13), while higher concentrations of paclitaxel (5 μM or more; Figure 2, lanes 15–19) inhibited the catalytic activity of Topo II, thus preventing the DNA relaxation.

Paclitaxel–DNA binding

Alteration in the local structure of DNA by drugs like distamycin has been known to modulate the effects of Topo II.[14] Like distamycin, binding of paclitaxel to DNA could lead to alteration of the local DNA

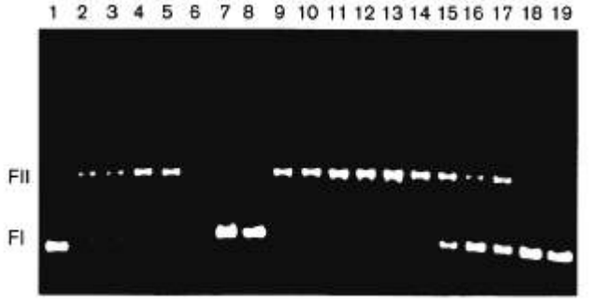

Figure 2. Dual effects of distamycin and paclitaxel on relaxation of supercoiled plasmid DNA, pBR322, by Topo II. A representative gel photograph is shown displaying the relaxation pattern of 0.2 μg supercoiled DNA by Topo II in the presence of varying concentrations of paclitaxel or distamycin (as a positive control). Gel was run overnight in 1 × TAE in the absence of ethidium bromide. Following the run, gel was stained with 0.5 μg/ml ethidium bromide for 1 h followed by destaining in deionized water for 30 min. Lane 1, supercoiled plasmid DNA substrate; lanes 2 and 9, solvent (DMSO) controls; lanes 3–8, 0.1, 0.5, 1, 5, 10 and 100 μM distamycin; lanes 10–19, 0.001, 0.01, 0.1, 0.5, 1, 5, 10, 50, 100 and 200 μM paclitaxel. FI, form I, negatively supercoiled DNA; FII, form II, nicked circular plasmid DNA.

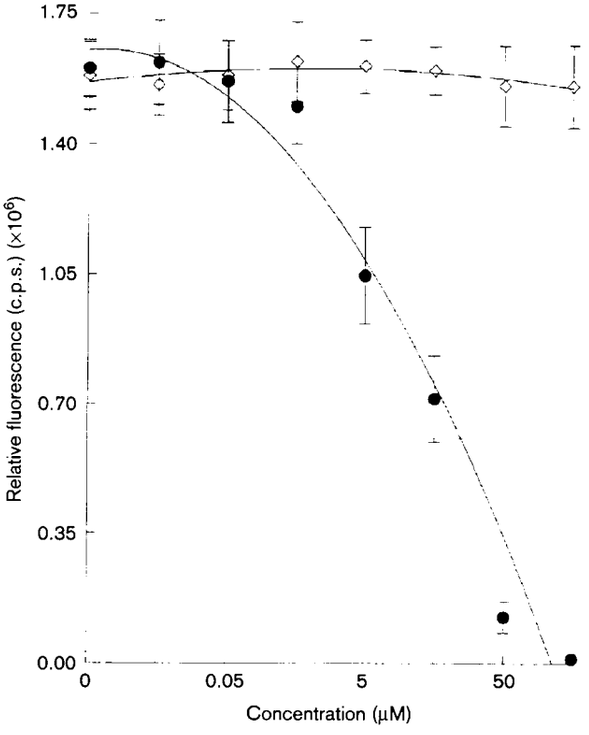

Figure 3. Paclitaxel neither intercalates nor binds to the minor groove of DNA. A fluorescence quenching assay, as described in Materials and methods, was used to study the interaction between paclitaxel and DNA. Increasing concentrations of paclitaxel (diamonds) or *m*-AMSA (circles, a positive control) were added to samples containing 10 μg calf thymus DNA and 1 μg/ml Hoechst 33258 in 1 × TNE. Fluorescence signals were monitored at 455 nm (emission maxima) with excitation wavelength set at 365 nm.

structure and this alteration may modulate the accessibility of Topo II that may ultimately be responsible for mediating paclitaxel's observed dual effects on Topo II. This postulate was tested by determining if paclitaxel can displace Hoechst 33258 dye (a minor groove binder that intercalates into DNA) from its binding site. Hoechst 33258 exhibits an enhanced fluorescence emission when bound to DNA as compared to its unbound (free) state. In the presence of a DNA-interacting drug, displacement of bound dye by the drug leads to the attenuation of emission signal. If indeed paclitaxel does directly bind to DNA, any dislodging of dye by paclitaxel should lead to a decrease in dye-induced fluorescence. Results depicted in Figure 3 clearly show that paclitaxel neither intercalates nor binds to the minor groove of DNA as indicated by no significant attenuation of fluorescent signal. In contrast, *m*-AMSA, a known intercalator, was very effective in displacing the fluorophore, leading to a rapid decline in fluorescence signal.

Paclitaxel interacts with Topo II

Our Hoechst 33258 displacement studies indicated that paclitaxel neither intercalates nor binds to the minor groove of DNA, suggesting that paclitaxel's effects on Topo II are not mediated by binding to DNA. In order to determine if the effects of paclitaxel are due to its interaction with Topo II, we utilized a titration study adopted from Tanabe *et al.*[15] The effect of increasing concentrations of enzyme or DNA was evaluated on an inhibitory concentration (50 μM) of paclitaxel in a relaxation assay. As shown in Figure 4, paclitaxel-mediated inhibition of Topo II was gradually titrated out by an increasing concentration of Topo II (Figure 4, lanes 4–9). A complete recovery of the relaxation activity of Topo II was attained by 5 U of Topo II (Figure 4, lane 6). Conversely, increasing concentrations of DNA had no effect on paclitaxel-mediated Topo II inhibition (data not shown). These observations, along with our DNA-binding studies, suggest that the modulation of Topo II's activity by paclitaxel might be the result of a direct interaction between paclitaxel and the enzyme.

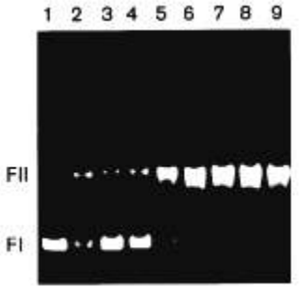

Figure 4. Increasing concentrations of Topo II can overcome paclitaxel-mediated Topo II inhibition. The relaxation pattern of 0.2 μg supercoiled DNA was monitored in the presence of increasing concentrations of Topo II and 50 μM paclitaxel. Gel was run and processed as for other relaxation assays. Lane 1, supercoiled DNA substrate; lane 2, solvent (DMSO) control; lanes 3–9, 1, 2, 4, 5, 6, 7 and 8 units of Topo II in the presence of 50 μM paclitaxel. FI, form I, negatively supercoiled DNA; FII, form II, nicked circular plasmid DNA.

Discussion

The results described here indicate that paclitaxel, a classical antimicrotubule agent, can modulate the catalytic activity of purified Topo IIα in a unique and dual fashion. In contrast to prototypical Topo II poisons, paclitaxel modifies the catalytic action of Topo II in a concentration-dependent manner, increasing it at lower concentrations while having an opposite effect at higher concentrations. The observed interaction of paclitaxel with Topo II, even if of secondary nature, may help to explain the excellent activity of paclitaxel in cancer cells.

There are three possible mechanisms by which anti-topoisomerase drugs act. In the first instance, a drug like *m*-AMSA can become part of the Topo II-DNA complex by interaction with DNA without having any effect on Topo II.[16] For paclitaxel, such a possibility is likely ruled out by the fact that paclitaxel is known to have little or no affinity for DNA,[1] an observation substantiated by our paclitaxel-DNA binding studies. This excludes the possibility of any local changes in DNA structure caused by paclitaxel that may alter Topo II's accessibility, a phenomenon presumably responsible for distamycin's dual effect.[14] Secondly, drugs like aclarubicin act as anti-topoisomerase drugs by interfering with initial DNA-Topo II complex formation without having any effect on DNA or Topo II alone.[17] In our titration studies, if paclitaxel does bind to DNA-Topo II complexes, we would have seen its inhibitory activity titrated out by both DNA and Topo II. However, it was affected by only increasing concentrations of enzyme, thus implying that the reported action of paclitaxel cannot be attributed to its effect on DNA-Topo II complexes. Thirdly, a drug such as ellipticine mediates its effect by directly binding to Topo II.[18] Our titration studies strongly suggest that paclitaxel is most likely to modulate Topo II activity by this mechanism.

Although various drugs are known to bind Topo II to mediate their effects, they elicit their response by diverse modes of action. For example, bisdioxopiperazines have been shown to inhibit Topo II directly by trapping the enzyme in a closed-clamp conformation that cannot transcend to its next dynamic form.[19] Ellipticine, an intercalative alkaloid, and etoposide, a non-intercalative epipodophyllotoxin, belong to the other side of the spectrum in that they enhance the forward rate of Topo II-mediated DNA cleavage by binding to Topo II.[18,20] Such diverse routes indicate that Topo II's catalytic activity may be altered by various known and lesser-known mechanisms. In this respect, paclitaxel may represent the prototype of a class of molecules which, in addition to interacting with microtubules, also contains a pharmacophoric moiety that alters the activity of Topo II, and possibly even other DNA binding proteins, in an unusual fashion with little information about its exact mechanism at present. This speculation gains credence in light of the fact that modifications of some antimicrotubule drugs in an effort to develop more potent agents have led to agents with anti-topoisomerase activity.[2,21] Also, it is noteworthy that like paclitaxel, Topo II inhibiting bis(2,6-dioxopiperazine) derivatives, in addition to being most effective during G_2/M phase of the cell cycle, also cause the formation of multiple abnormal asters,[22] a classical feature seen in cells exposed to paclitaxel.[1] Although these derivatives were shown to have no effect on microtubules, this similarity is strikingly intriguing.

The uniqueness of the observed paclitaxel-mediated alteration of Topo II's activity lies in its concentration-dependent opposing effects, whereby at lower concentrations paclitaxel increases the catalytic efficacy of Topo II while inhibiting it at higher concentrations. One possible mechanism that may help explain the dual action of paclitaxel on Topo II is the likelihood of the existence of two (or more) discrete paclitaxel binding domains on Topo II, that when occupied, can have contrasting effects on Topo II's activity. At lower concentrations of paclitaxel, there may be an allosteric effect whereby paclitaxel molecules bind to certain sites on Topo II leading to an increased catalytic efficacy of Topo II (increased turnover rate). At higher, inhibitory concentrations, paclitaxel may either block the catalytic site of the enzyme directly or, like

bisdioxopiperazines, 'freeze' the enzyme molecule in a non-functional conformation. Such so-called 'functional drug-interaction domains' have been shown to exist for other Topo II inhibitors/poisons.[23] Presently, we are conducting studies to elucidate if these functional domains overlap with the putative domains occupied by paclitaxel. Although highly speculative, physico-chemically, a hydrophobic enzyme like Topo II[24] may have potential sites for non-covalent interactions with paclitaxel, another hydrophobic molecule. These sites, if present, could be one of the physical locations corresponding to the above-mentioned functional domains.

In addition to its effect on microtubules, paclitaxel reported dual effect on Topo II can have important consequences. The lower Topo II *stimulating* concentrations of paclitaxel may lead to illegitimate recombinational events that may contribute to ultimate cell death by starting the process of cell demise earlier than the manifestation of paclitaxel's effect on microtubules. On the other hand, higher paclitaxel concentrations, in addition to microtubule stabilization, may further halt cell division by inhibition of Topo II, an enzyme required for proper chromosomal segregation.[2] Such multiple effects may thus explain the excellent antitumor activity of paclitaxel.

It should be emphasized that the paclitaxel concentrations (including inhibitory concentrations) used in this study are well within the range achievable intracellularly. Jordan *et al.* have reported earlier that a 20 h exposure of cells to 1 μM paclitaxel (a clinically achievable concentration, reviewed by Rowinsky[25]) results in intracellular concentrations as high as 240 μM,[26] a concentration well above the inhibitory concentrations used in our study. Due to the variable amount of Topo II activity present in 1 μl of purified enzyme, one critical factor in our decatenation and relaxation assay studies was the careful titration of enzyme concentrations to observe the reported effects of paclitaxel on Topo II. The described effects of paclitaxel on Topo II were also confirmed using Topo II-containing nuclear extracts isolated from xrs-6 cells (unpublished observation).

In summary, these studies indicate that besides microtubules, paclitaxel may interact with Topo II in a manner that disrupts the enzyme's activity and such an interaction could be an important contributory factor towards the sum total of paclitaxel's cytotoxic effects, thus explaining its excellent activity as compared to other spindle poisons. Intense investigations are currently underway to determine the mechanism for paclitaxel's dual effects on Topo II. Additionally, our studies also suggest the presence of a Topo II-interacting pharmacophore in the paclitaxel molecule. Elucidation of this putative pharmacophoric moiety could be helpful in designing structural analogs of paclitaxel that may retain their antimicrotubule activity while simultaneously modifying the action of Topo II (and possibly other DNA binding proteins), thus eliciting their cytotoxic action by multiple mechanisms and increasing their efficacy in killing cancer cells.

Acknowledgments

We are grateful to Dr Marvin K Schulte for his critical review of the manuscript and for helpful discussions.

References

1. Horwitz SB. Paclitaxel (Taxol): mechanism of action. *Ann Oncol* 1994; **5** (suppl. 6): S3-6.
2. Pommier Y, Fesan MR, Goldwasser F. Topoisomerase inhibitors. In: Chabner BA, Longo LA, eds. *Cancer chemotherapy and biotherapy*, 2nd edn. Philadelphia: Lippincott-Raven 1996: 435-61.
3. Boege F. Analysis of eukaroytic DNA topoisomerase and topoisomerase-directed drug effects. *Eur J Clin Chem Biochem* 1996; **34**: 873-88.
4. Swaffar DS, Ireland CM, Barrows LR. A rapid mechanism-based screen to detect potential anti-cancer agents. *Anti-Cancer Drugs* 1994; **5**: 15-23.
5. Jeggo PA, Sedgwick SG, Kemp LM. X-ray sensitive mutants of CHO cells defective in double strand break rejoining. *Mutat Res* 1983; **112**: 313-27.
6. Barrows LR, Borchers AH, Paxton MB. Transfectant CHO cells expressing O^6-alkylguanine-DNA-alkyltransferase display increased resistance to DNA damage other than O^6-guanine alkylation. *Carcinogenesis* 1987; **8**: 1853-9.
7. Jeggo PA, Caldecott K, Pidsley S, Banks GR. Sensitivity of Chinese hamster ovary mutants defective in DNA double strand break repair to topoisomerase II inhibitors. *Cancer Res* 1989; **49**: 7057-63.
8. Warters RL, Lyons BW, Li TM. Topoisomerase II activity in a DNA double strand repair deficient Chinese hamster ovary cell line. *Mutat Res* 1991; **254**: 167-74.
9. Povirk LF. DNA damage and mutagenesis by radiomimetic DNA-cleaving agents: bleomycin, neocarzinostatin and other enediynes. *Mutat Res* 1996; **355**: 71-89.
10. Barrows LR, Radisky DC, Swaffar DS, Copp BR, *et al.* Makaluvamines, marine natural products, are active anticancer agents and DNA topo II inhibitors. *Anti-Cancer Drug Des* 1993; **8**: 333-47.
11. Taccioli GE, Rathbun G, Oltz E, Stamato T, *et al.* Impairment of V(D)J recombination in double-strand break repair mutants. *Science* 1993; **260**: 207-10.
12. Osheroff N, Shelton ER, Brutlag DL. DNA topoisomerase II from *Drosophila melanogaster*: relaxation of supercoiled DNA. *J Biol Chem* 1983; **258**: 9536-43.
13. Woynarowski JM, McHugh M, Sigmund RD, Beerman TA. Modulation of topoisomerase II catalytic activity by DNA minor groove binding agents distamycin, Hoechst 33258 and 4',6-diamidine-2-phenylindole. *Mol Pharmacol* 1989; **35**: 177-82.

14. Fesan M, Pommier Y. Mammalian topoisomerase II activity is modulated by the DNA minor groove binder distamycin in simian virus 40 DNA. *J Biol Chem* 1989; **264**: 11354–9.
15. Tanabe K, Ikegami Y, Ishida R, Andoh T. Inhibition of topoisomerase II by antitumor agents bis(2,6-dioxopiperazine) derivatives. *Cancer Res* 1991; **51**: 4903–8.
16. Waring MJ. DNA modification and cancer. *Annu Rev Biochem* 1981; **50**: 159–92.
17. Sorensen BS, Sinding J, Anderson AH, Alsner J, *et al.* Mode of action of topoisomerase II-targeting agents at a specific DNA sequence. Uncoupling the DNA cleavage and religation events. *J Mol Biol* 1992; **228**: 778–86.
18. Froelich-Ammon SJ, Patchan MW, Osheroff N, Thompson RB. Topoisomerase II binds to ellipticine in the absence or presence of DNA. *J Biol Chem* 1995, **270**: 14998–5004.
19. Roca J, Ishida R, Berger JM, Andoh T, *et al.* Antitumor bisdioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp. *Proc Natl Acad Sci USA* 1994; **91**: 1781–5.
20. Burdan DA, Kingma PS, Froelich-Ammon SJ, Thompson RB, *et al.* Topoisomerase II–etoposide interactions direct the formation of drug induced enzyme-cleavage complexes. *J Biol Chem* 1996; **271**: 29238–44.
21. Guan J, Zhu XK, Tachibana Y, Bastow KF, *et al.* Antitumor agents. 185. Synthesis and biological evaluation of tridemethylthiocolchicine analogs as novel topoisomerase II inhibitors. *J Med Chem* 1998; **41**: 1956–61.
22. Ishida R, Tetsuo M, Narita T, Yui R, *et al.* Inhibition of intracellular topoisomerase II by antitumor bis(2,6-dioxopiperazine) derivatives: mode of cell growth inhibition distinct from that of cleavable complex-forming type inhibitors. *Cancer Res* 1991; **51**: 4909–16.
23. Osheroff N, Corbett AH, Elsea SH, Westergaard M. Defining functional drug-interaction domains on topoisomerase II by exploiting mechanistic differences between drug classes. *Cancer Chemother Pharmacol* 1994; **34**: S19–25.
24. Halligan BD, Edwards KA, Liu LF. Purification and characterization of a type II DNA topoisomerase from bovine calf thymus. *J Biol Chem* 1985; **260**: 2475–82.
25. Rowinsky EK, Donehower RC. Antimicrotubule agents. In: Chabner BA, Longo DL, eds. *Cancer chemotherapy and biotherapy*, 2nd edn. Philadelphia: Lippincott-Raven 1996: 263–96.
26. Jordan MA, Wendall K, Gardiner S, Wilson L, *et al.* Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. *Cancer Res* 1996; **56**: 816–25.

(Received 22 December 1998; accepted 8 January 1999)