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COMMENTARY

DNA TOPOISOMERASES AS TARGETS FOR CANCER THERAPY

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The recent demonstration that the nuclear enzyme DNA topoisomerase II mediates DNA scission in cells exposed to certain classes of anticancer agents creates important new opportunities for understanding how the drugs work. In turn, the drugs provide much needed new tools for probing the actions of this unusual enzyme in mammalian systems. More philosophically, the emergence of this concept offers yet another satisfying reassurance that pharmacology and biology are truly symbiotic and interdependent. In this commentary, I shall briefly review the data which support this novel drug–enzyme interaction and then indicate some of its potential implications.

In most biological systems, DNA behaves as though it is topologically constrained, i.e. as if the two ends of the molecule are fixed in space. This property of DNA allows for higher orders of structure which can dictate biological function. For example, if the number of times two DNA strands are intertwined in a topological domain is diminished (i.e. the domain is underwound), strand separation is favored, and such a phenomenon would facilitate processes such as DNA replication, transcription, transposition, and viral integration. Regulation of DNA topology is, thus, of critical importance to normal cellular function, and one of the principal means by which the cell accomplishes this is the DNA topoisomerases (see Refs. 1 and 2 for reviews). These enzymes may be broadly and simply defined as proteins which catalyze the breaking and rejoining of DNA strands in concert. In eukaryotic systems, there are two distinct groups of topoisomerases. Type I topoisomerase is an enzyme of approximately 100,000 molecular weight and is defined by its ability to break and then reseal a single DNA strand. The enzyme remains bound at the 3′ end of the break site during this process. It is this covalent phosphotyrosine linkage between enzyme and DNA which conserves the energy of the phosphodiester bond and facilitates rejoining. By virtue of its ability to break and rejoin DNA, type I topoisomerases are able to relax supercoiled double-stranded DNA [3], promote the linking of two complementary single-stranded DNA rings into a fully relaxed duplex ring [4] and allow catenation of double-stranded DNA circles providing that at least one of the circles is nicked [5]. The biological role of topoisomerase I is poorly understood. Studies in bacterial mutants suggest that its primary role is the modulation of overall superhelical density in chromosomal DNA [6, 7]. Perturbations at this density have important effects on transcriptional processes [6, 7]. It likely plays a similar role in higher species, but there has been little definitive published evidence on this point to date. The relative merit of type I topoisomerase as a target for antitumor agents is largely unknown. It is noteworthy, however, that Hsiang and Liu [8] have demonstrated recently that camptothecin-induced DNA damage is likely mediated by a type I topoisomerase. This plant alkaloid demonstrated antitumor activity in early studies but was eventually dropped from development because of toxicity. If the data of Hsiang and Liu prove correct with respect to relating topoisomerase I to the antitumor activity of camptothecin, it may be an opportune time to reconsider this compound or one of its congeners.

There is a great deal more evidence suggesting the importance of type II topoisomerase as a target for cancer therapy. This enzyme is composed of homologous dimers weighing approximately 170 kilodaltons each [1, 2]. It is characterized by the ability to transiently break and reseal both DNA strands simultaneously, allowing the passage of a separate double helical strand through the break site. Type II topoisomerase also binds to free DNA ends during the breakage–reunion process by virtue of phosphotyrosine linkage. In contrast to the type I enzyme, however, it remains covalently bound at the 5′ end of the DNA break site and is only loosely associated with the 3′ end (Fig. 1). There are really two components to the action of type II topoisomerase. The first is the ability to concurrently and reversibly cleave both strands of DNA, and the second is the so-called "strand passing" or catalytic activity. This latter action is ATP dependent. As a result of these actions, there are three principal reactions catalyzed by type II topoisomerase [9–11]. They are: (a) the ability to remove complex knots from topologically constrained DNA, (b) the ability to remove, or, in the case of DNA gyrase, create supercoiling in topologically constrained DNA, and (c) the ability to reversibly catenate double-stranded DNA circles. The biological role of type II topoisomerase is only now beginning to emerge. Studies in a *Saccharomyces cerevesiae* temperature-sensitive topoisomerase mutant indicate that, at nonpermissive temperatures, the yeast arrests at the completion of S phase [12]. Examination of closed circular plasmid-like DNA structures in this yeast indicates failure to segregate daughter DNA molecules at the completion of replication. This is precisely what would be expected from the loss of a decatenating activity, such as is found in topoisomerase II. Supporting the

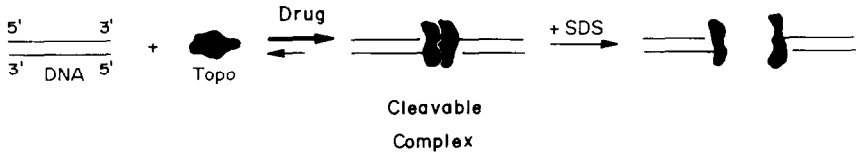

Fig. 1. Reaction mechanism for DNA cleavage by topoisomerase II. The reaction is shifted to the right in the presence of certain intercalating agents or epipodophyllotoxins. Detergents such as SDS denature the protein dimer, revealing the protein associated DNA break.

importance of the enzyme for DNA replication is the observation by Noguchi *et al.* [13] that topoisomerase II is a component of "replitase," a multienzyme complex which appears to direct DNA replication. There is also evidence that topoisomerase II may act in concert with topoisomerase I in controlling DNA transcription in frog oocytes [14]. As one might predict from the presumed importance of topoisomerase II in regulating activities of DNA, the enzyme is closely associated with the nuclear matrix in interphase and appears to be a major structural protein of mitotic chromosomes [15, 16].

There is ample precedence for considering DNA topoisomerases as targets for chemotherapeutic agents. Indeed, much of the early characterization of DNA gyrase was based on its interaction with the antibiotics novobiocin and nalidixic acid. Gyrase is a form of topoisomerase II found only in bacterial systems which, in addition to the properties already described, also has the ability to introduce supercoils into topologically relaxed DNA [17]. From the gyrase studies has evolved the concept that novobiocin blocks the strand passing activity by binding to a particular subunit of the enzyme but does not induce cleavage of DNA [18]. On the other hand, nalidixic acid binds to a different subunit in such a way as to shift the reaction demonstrated in Fig. 1 markedly to the right, causing the formation of a so-called "cleavage complex" which, upon exposure to a detergent such as sodium dodecyl sulfate (SDS), results in DNA double-strand breaks with the enzyme being bound at the 5′ terminus of the break site [19]. Genetic mapping studies indicate that, when a nalidixic acid resistant gyrase mutant is hybridized with a drug-sensitive wild type cell to form a partial bacterial diploid, drug sensitivity is dominant [20]. This result implies that it is the formation of the cleavable complex and not the subsequent loss of topoisomerase activity which is responsible for lethality. There is a growing body of evidence that the interaction of certain anticancer agents with mammalian topoisomerase II exhibits most of the important features of the nalidixic acid–DNA gyrase model.

The first suggestion that DNA topoisomerase II might be involved in the action of anticancer agents came from studies of the intercalating agents adriamycin and ellipticine [21, 22]. When mouse leukemia cells are exposed to these agents and then examined by the DNA alkaline elution technique, a unique type of DNA strand break is observed. This strand break, termed a protein-associated DNA break, appears to have a protein tightly, if not covalently, bound to the DNA in a spatial and stoichiometric relationship which suggests that the protein is bound at or near the break site. From these data, the investigators hypothesized that the topological perturbations imposed by DNA intercalation may have elicited a DNA topoisomerase to break the DNA but that, in the presence of drug, the enzyme could not reseal the break. This hypothesis proved only partially correct. Subsequently, Ross and Bradley [23] demonstrated that many of these protein-associated DNA breaks were actually double-stranded breaks, hinting that if a topoisomerase were involved, it may be topoisomerase II. This observation was extended to other intercalating agents by Zwelling *et al.* [24, 25]. Protein-associated DNA breaks were later shown to be mediated by a salt extractable nuclear protein [26], and inferential evidence indicated that a protein was, indeed, bound at the 5′ DNA terminus as would be predicted for a topoisomerase II [27]. The actual demonstration, however, that intercalating agents could promote DNA cleavage by topoisomerase II was provided by Liu's group using highly purified enzyme from calf thymus [28–30]. These investigators observed that, in the presence of a variety of intercalating agents, site specific DNA cleavage by topoisomerase II was markedly stimulated and that the enzyme remained bound at the 5′ end of the cleavage site as predicted. They also observed that the drugs could inhibit the strand passing activity of topoisomerase II but that drug potency with regard to this action did not always parallel that for cleavage activity. More recently, this group has shown that the protein-associated DNA breaks created intracellularly could be immunoprecipitated with an anti-topoisomerase II polyclonal antibody [31]. Interestingly, Liu's *in vitro* studies established that drug-stimulated DNA cleavage by topoisomerase II did not require a topologically restrained substrate. This suggested that the interaction with the enzyme did not depend on the topological changes which result from the intercalative mode of DNA binding. This point was later confirmed in studies on another group of antitumor agents, the epipodophyllotoxins, a semisynthetic series of podophyllotoxin congeners which include etoposide (VP-16) and teniposide (VM-26). These compounds have been shown to cause both single and double DNA strand breaks and DNA protein cross-links in mammalian cells by a temperature-sensitive nuclear process [32, 33]. As was the case with the intercalating agents, the drug-induced strand break activity was dependent on a heat labile, salt extractable nuclear protein and was stimulated by ATP [34]. The epipodophyllotoxins were subsequently shown to inhibit strand passing activity and

potently induce DNA cleavage by topoisomerase II *in vitro* [35, 36]. Interestingly, equilibrium dialysis experiments indicated that etoposide, unlike the intercalating agents, did not bind to DNA, thus strengthening the argument that DNA binding is not an essential requirement for drugs to elicit topoisomerase-mediated DNA cleavage [35]. Thus, in summary, there is a substantial body of evidence which indicates that protein-associated DNA breaks resulting from intercalating agents and epipodophyllotoxins are mediated through an interaction between drug and topoisomerase II resulting in enzymatic cleavage of DNA.

The relationship between these unique topoisomerase-mediated DNA breaks and antitumor effect has been more difficult to convincingly document. Several early papers suggested that there was little or no relationship between drug potency with respect to DNA cleavage and cytotoxicity when comparing different intercalating agents [25, 37]. Some of the difficulty arose because drugs of vastly different structures were being compared, and factors other than DNA cleavage were clearly important. For instance, although ellipticine causes a much higher frequency of DNA single- and double-strand breaks than adriamycin, it is far less potent in cytotoxicity assays [37]. This, however, probably results from the fact that ellipticine-induced breaks are rapidly repaired in cells when drug is removed from the surrounding medium, while adriamycin-induced breaks are retained much longer [38]. When structurally more similar groups of congeners have been studied, a much better relationship has been demonstrated between DNA cleavage and cytotoxicity. For instance, the aminoacridines *o*- and *m*-AMSA [4′-(9-acridinylamino)methanesulfor-*m*-anisidide] are quite distinctive in that the latter is far more potent in causing DNA cleavage and antitumor effect [24, 39]. Studies *in vitro* demonstrate that it is only the meta isomer which induces topoisomerase-mediated DNA cleavage [28]. Long *et al.* [40] have examined a large series of epipodophyllotoxin congeners in a human lung adenocarcinoma cell line and found a very close relationship between ability to produce DNA breakage intracellularly and cytotoxicity. In a somewhat different approach to defining the relationship between topoisomerase-mediated DNA cleavage and cytotoxicity, Rowe *et al.* [41] seized upon the observation that the intercalating dye ethidium bromide actually blocks topoisomerase-mediated DNA cleavage at high concentrations. Presumably this is a result of creating topological distortion in the DNA, thus interfering with enzyme recognition of DNA binding sites. They found that, when mammalian cells were exposed simultaneously to etoposide and ethidium bromide, there was a progressive reduction in etoposide-induced DNA strand scission and cytotoxicity as the concentration of ethidium bromide was increased. These effects could not be accounted for by alterations of intracellular drug concentration or by virtue of inhibition of macromolecular synthesis. Furthermore, the effect was observed only when ethidium bromide was present at the time that the epipodophyllotoxin was added. Thus, there is strong circumstantial evidence that DNA cleavage correlates with cytotoxicity but no direct proof. A most likely source for such proof will be mutant cell lines which are resistant to the topoisomerase-mediated drugs. One candidate is the Vpm-5 Chinese hamster ovary line isolated by Gupta [42] following a brief exposure to the epipodophyllotoxin teniposide. This cell line exhibits significant cross-resistance to etoposide, adriamycin, daunomycin, *m*-AMSA and mitoxantrone, all agents thought to act through topoisomerase [42, 43]. It is not a transport mutant but does demonstrate a marked reduction in DNA cleavage following exposure to these drugs [43]. Preliminary evidence indicates that topoisomerase-mediated DNA cleavage activity in a crude nuclear extract from this cell line is reduced, but whether this is a qualitative or quantitative topoisomerase alteration is, at present, undefined (unpublished observation). A second cell line, recently described by Pommier *et al.* [44], may also represent a topoisomerase variant. Interestingly, a somatic cell hybrid formed between Vpm-5 and the wild type parental line demonstrates nearly complete reconstitution of drug sensitivity with respect to both DNA cleavage [43] and cytoxicity [42]. The fact that drug sensitivity is dominant is consistent with the experiments previously cited in nalidixic-resistant bacteria [20] and again indicates that it is the cleavable complex formation which is lethal and not inhibition of topoisomerase catalytic function. Additional support for this latter point comes from the fact that *o*-AMSA and ethidium bromide are quite effective at blocking catalytic activity but do not stimulate topoisomerase-mediated DNA cleavage and are only weakly cytotoxic [24, 28, 29, 43].

The putative role of topoisomerase in mediating antitumor activity is a somewhat novel one in cancer pharmacology, namely that the drug action does not result from blocking a normal enzyme function but rather by subverting it in such a way as to render the enzyme a lethal instrument. In a sense, the enzyme becomes a required co-factor for drug action. Two important questions are raised by this concept. First, why is the cleavable complex lethal, i.e. what are the biochemical consequences, and how do they result in cell death? Second, how can this concept be used to improve the efficacy of cancer therapy?

There is little information in mammalian cells regarding the mechanism by which the cleavable complex results in cell death. The DNA breaks are generally rapidly reversed following drug removal [24, 32], but it would appear that either the repair is not complete or that unknown events consequent to the breaks result in more lasting forms of DNA damage. Following exposure to epipodophyllotoxins or intercalating agents, cells commonly arrest in the G_2 phase of the cell cycle [45, 46], a phenomenon frequently observed with drugs that cause DNA damage. This may be a time when cells attempt to repair damage prior to mitosis. In this regard, it is interesting that caffeine has been shown to increase the cytotoxicity of several intercalating agents and epipodophyllotoxins [37, 47]. While the mechanism of this potentiation has not been clearly demonstrated, it has been proposed that caffeine may prevent the G_2 arrest causing cells to enter mitosis without having completed the necessary DNA repair

[48]. Further evidence that the cleavable complex causes lasting DNA damage is the observation of chromosomal aberrations following exposure to a variety of intercalating agents and epipodophyllotoxins [49, 50]. It is somewhat difficult to imagine what repair pathways might be involved in cellular recovery from topoisomerase-mediated DNA damage. Zwelling and Mattern [51] studied the effects of *m*-AMSA in a limited number of cell lines from patients with either xeroderma pigmentosa or ataxia telangectasia and found no difference in DNA cleavage or cytotoxicity when compared to normal human fibroblasts. It would seem that examination of a broader range of repair deficient cell lines might be rewarding.

The identification of topoisomerase II as the intracellular target for certain anticancer agents makes it imperative to determine how intracellular enzyme content affects drug action. Several lines of evidence suggest that topoisomerase II activity is not constant but may fluctuate with the proliferative status of the cell. Duguet *et al.* [52] have shown that topoisomerase II activity is markedly stimulated in the nuclei of regenerating cells following partial hepatectomy. This same group subsequently demonstrated that topoisomerase II activity increased in nuclear extracts of mitogen-stimulated guinea pig lymphocytes [53]. This increase was temporally coincident with stimulation of DNA synthesis. Miskimins *et al.* [54] examined topoisomerase II activity in human and mouse fibroblasts following stimulation by epidermal growth factor. Enzyme activity again increased in parallel with DNA synthesis. None of these studies actually examined topoisomerase protein content, and it is unclear whether these effects are due to qualitative or quantitative changes in topoisomerase II. In this regard, it is important to note that topoisomerase II is subject to phosphorylation by both tyrosine [55] and serine [56] kinases, and, thus, activity may not be reflected in enzyme content. An increase in enzyme activity of topoisomerase I was observed by Chow and Pearson [57] following infection of mammalian cells by adenovirus. They postulated that this was a response to expression of a gene in the early region IA (E1A) of the viral genome. Topoisomerase II content was not examined. Since the E1A gene product is thought to play a crucial role in the process by which adenovirus transforms mammalian cells, this is clearly an important area for future investigation. There are two preliminary reports which indicate that topoisomerase-mediated DNA cleavage and cytotoxicity by intercalating agents and epipodophyllotoxins may be reduced markedly in nonproliferating normal cells [58, 59]. Interestingly, in both reports it was noted that there was much less of a decrease in drug activity in quiescent transformed cells, suggesting again that topoisomerase activity may be regulated differently in the transformed state. It is unknown whether the decrease in topoisomerase activity was due to decreased enzyme content or post-translational modification of the enzyme. Whichever proves to be the case, the observation that there are mechanisms for altering topoisomerase activity and that such changes may influence drug sensitivity raises the possibility of improving therapeutic index if a means could be found of stimulating enzyme activity in malignant but not normal cells. Zwelling *et al.* [60] have shown, for instance, that estrogen stimulation of hormonally dependent human breast cancer cells increased DNA scission by *m*-AMSA and the anthracycline 5-iminodaunorubicin. While they did not measure topoisomerase activity or content, this observation suggests the kind of therapeutic maneuvers which might be possible once the topoisomerase mechanism is better understood. Further studies of the mechanism for controlling topoisomerase II activity are imperative.

Another promising byproduct of topoisomerase research may be the identification of new classes of anticancer drugs directed at this enzyme. Considering the rather broad spectrum of activity represented by anthracyclines, aminoacridines, anthracenediones and epipodophyllotoxins, there would seem to be a high likelihood that other drugs which induce formation of the cleavable complex would be clinically effective as well. Indeed, it may be interesting to re-evaluate a number of nalidixic acid congeners which were developed for antibacterial use but proved too toxic to mammalian cells. Some of these may prove to be inhibitors of mammalian topoisomerase II. Another avenue worthy of exploration is the development of novobiocin-like compounds which block catalytic activity by binding to enzyme but do not induce DNA cleavage. Novobiocin itself is only weakly inhibitory towards mammalian DNA topoisomerase II, but there may be congeners which would prove more potent.

Finally, there is reason to believe that studies using topoisomerase-mediated drugs may yield important information about the enzyme itself. For example, Yang *et al.* [31] have demonstrated that, when SV-40 virus infected monkey cells were treated with *m*-AMSA, topoisomerase-mediated double-strand breaks were formed in the viral chromatin. A major topoisomerase cleavage site was induced during late viral infection and was mapped to the DNAase I hypersensitive region. This enhanced cleavage may represent changes in chromatin structure which allow for greater topoisomerase access or may imply that the enzyme itself has a role in determining the sensitivity of this site to nuclease action. Another more preliminary report indicates that DNA sequences commonly associated with Z DNA may be non-randomly associated with *m*-AMSA-induced DNA cleavage sites [61]. In a somewhat different vein, it may be possible to use the topoisomerase-mediated drugs to generate temperature-sensitive topoisomerase mutant cell lines which would be invaluable for study of the function of this enzyme.

In summary, DNA topoisomerase II has become a focal point of research for individuals in areas as diverse as nucleotide mapping, cell biology, and cancer chemotherapy. It is highly likely that these studies will have substantial rewards, both at the bench and at the bedside. If the preliminary evidence with camptothecin substantiates a role for topoisomerase I as well, then unraveling the topological mysteries of DNA may become even more profitable.

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