Unknotting the Complexities of Multidrug Resistance: The Involvement of DNA Topoisomerases in Drug Action and Resistance

William T. Beck

Topoisomerases are essential nuclear enzymes that catalyze the interconversion of topological forms of single- and double-stranded DNA; in doing so, they are intimately involved in processes related to cell growth and division and appear to be structural and functional components of the cell nucleus (*1-3*). Over the past several years, there has been considerable interest in these enzymes as targets of certain classes of antineoplastic agents, including camptothecin, doxorubicin and daunorubicin (anthracyclines), teniposide and etoposide (epipodophyllotoxins), and amsacrine (an aminoacridine) (*3-5*). While camptothecin and analogs appear to be specific inhibitors of DNA topoisomerase I (*6*), the other drugs inhibit the activity of DNA topoisomerase II (*7,8*).

Rodent and human tumor cell lines expressing resistance to drugs that act on topoisomerase I (*9,10*) or topoisomerase II (*11-13*) have been described, and resistance, where it has been studied, appears to be associated with decreased activity of these enzymes (*9,10,14-16*). It is of interest that cells selected for resistance to one inhibitor of topoisomerase II express cross-resistance to other classes of drugs that interact with this enzyme, a form of multidrug resistance (MDR) (*13,14*). We have termed cells expressing this phenotype "at-MDR" (*16*), since the phenotype appears to be mediated by alterations in topoisomerase II. Because topoisomerase II-interacting drugs are used clinically, and because selection of cell lines expressing altered topoisomerases can be achieved relatively easily, it is likely that the tumor cells of patients who have been treated with these drugs will also develop an altered topoisomerase form of resistance to these agents. Accordingly, efforts to understand the biochemical and molecular bases of at-MDR are warranted.

While several recent biochemical studies have provided insights into at-MDR (*16-19*), the paper by Tan et al. in this issue of the Journal (*20*) poses new challenges to our understanding of this complex problem. I will confine my comments here mostly to topoisomerase II and at-MDR but will address camptothecin resistance and topoisomerase I where appropriate.

What do we know about the biochemical and molecular basis of at-MDR? In contrast to "classic" P-glycoprotein-associated MDR (Pgp-MDR), cells expressing "pure" at-MDR are generally unaltered in drug accumulation and retention (*13*) and do not overexpress P-glycoprotein (*21*). Insofar as we know, at-MDR cells all display decreased activities of topoisomerase II (*14-16,22*), but the precise mechanism(s) through which this decrease is mediated may vary. In thinking about the possible bases of decreased or altered topoisomerase II activity, it is instructive to consider the steps involved in the normal functioning of the enzyme. Osheroff (*23*) proposed that enzyme activity can be viewed as comprising several discrete steps: 1) binding/recognition of specific DNA sequences, 2) strand-cleavage and covalent attachment of the enzyme to the cleaved 5′ termini, 3) passage of the second strand of DNA, which requires adenosine triphosphate (ATP) binding but not hydrolysis, 4) religation of the cleaved DNA, and 5) enzyme turnover (catalysis), which requires ATP hydrolysis. A mutation in the topoisomerase gene could affect any or all of these steps and lead to a decrease in enzyme activity.

In many (*15,18,19*) but not all (*16*) at-MDR cell lines, decreased catalytic activities of, and cleavage by, DNA topoisomerase II are associated with decreased amounts of immunoreactive enzyme in nuclear extracts. We have found that nuclear matrix-bound topoisomerase II is decreased in amount and activity in our teniposide-resistant human CEM cell lines, compared to drug-sensitive cells (*24*). Further, we recently showed that the requirement of topoisomerase II for ATP is increased in resistant cells (*17*). Experiments with novobiocin and a nonhydrolyzable analog of ATP suggested that topoisomerase II from the resistant cells bound ATP less well than the enzyme from the sensitive cells, and we postulated that this phenomenon was due to a structural alteration or mutation in the gene encoding the enzyme (*17*), a possibility that we are presently examining. Based on the steps involved in topoisomerase activity, the proposed mutation in our cells most likely affects second-strand passage. Resistant topoisomerases have been purified from rodent (*18,19*) and human (*9,15*) cell lines and have been shown to differ from the sensitive cell enzymes in activity or physical properties. The finding of decreased amounts of topoisomerase II in resistant cell extracts is consistent with the fact that the phenotype associated with decreased topoisomerase activity is expressed recessively (*10,14,25,26*).

The paper by Tan et al. in this issue of the Journal presents two interesting and potentially important concepts regarding topoisomerases and the drugs that interact with them. The first is that apparently one allele for topoisomerase is shut down in the resistant cell lines, and the investigators have presented some data indicating that the basis for this may be hypermethylation of the topoisomerase genes. The other is that resistance to a topoisomerase II inhibitor confers collateral sensitivity to inhibitors of topoisomerase I and vice versa.

Received October 9, 1989; accepted October 10, 1989.

Department of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38101.

The idea that a possible mode of drug resistance may be by "down-regulation" of topoisomerase II via hypermethylation of the gene and expression of only one of the alleles is intriguing. Hypermethylation of topoisomerase II in HL-60/*m*-AMSA cells was also suggested in another recent study (*15*), although the possibility of a mutation could not be ruled out. While altered regulation by hypermethylation is consistent with the recessive nature of drug resistance associated with decreased topoisomerase activities, it differs from the idea that this type of resistance is due to mutations in the genes encoding these enzymes. Seemingly, hypermethylation may be another way of regulating topoisomerase II, and possibly topoisomerase I. It will be of interest to examine other possible modes of topoisomerase gene regulation in drug-resistant cells, such as altered rates of transcription. In the context of the Tan study, confirmation of hypermethylation in other at-MDR lines could open new avenues for therapeutic intervention. For example, treatment with azacitidine, which inhibits DNA methylation (*27*), might "up-regulate" the expression of topoisomerase II and render topoisomerase II-resistant (presumably hypermethylated) cells sensitive to antineoplastic agents.

The other concept to come from the paper by Tan et al. is that of the apparent reciprocal relationship between the two topoisomerase enzymes. Studies with yeast mutants suggest that topoisomerase I is *not* an essential enzyme, that these organisms can function without it (*28*), but that topoisomerase II may be able to assume some of its functions (*29*). Controls for the two enzymes in mammalian cells may differ from those in yeast. Sensitivity to the topoisomerase I inhibitor camptothecin has not been reported in other cell lines resistant to topoisomerase II inhibitors, but moderate hypersensitivity to topoisomerase II inhibitors was seen in a Chinese hamster ovary (CHO) cell line selected for high resistance to camptothecin (*10*). While decreased levels of topoisomerase I were shown in these camptothecin-resistant CHO cells, topoisomerase II levels did not appear to be elevated (*10*). In other studies, topoisomerase II levels were found to be increased in an ataxia telangiectasia fibroblast cell line (*30*), in a doxorubicin-hypersensitive CHO cell line (*31*), and in mechlorethamine-resistant Raji human Burkitt's lymphoma cells (*32*). The drug-resistant Raji cells were collaterally sensitive not only to several topoisomerase II inhibitors, but also to camptothecin (*32*). Although the ataxia telangiectasia fibroblast line expressed a reciprocal decrease in topoisomerase I levels, this effect was not seen in the doxorubicin-hypersensitive CHO line, and the authors concluded that decreased topoisomerase I is not inevitably linked with increased levels of topoisomerase II (*30*). Clearly, more studies are needed to determine whether a functional relationship between the two enzymes exists in mammalian cells.

It is now clear that "natural product" multidrug resistance can take several forms. Although much has been learned recently about that form associated with the overexpression of P-glycoprotein (*33,34*), we are just beginning to see the shape and magnitude of the problem of MDR associated with altered topoisomerase II. Resistance associated with altered topoisomerase I has not been studied extensively. It is presently unclear whether mutation or altered regulation of the enzymes is the predominant mechanism responsible for the resistant phenotype, but work in several laboratories should provide information about this in the near future. There are likely to be several different pathways that can lead to drug resistance via alterations in topoisomerase II activity and produce a similar phenotype, at-MDR. The paper by Tan et al. in this issue of the Journal affords new ways of thinking about the role of altered topoisomerases that may help us unknot the complexities of multidrug resistance.

References

(*1*) WANG JC: DNA topoisomerases. Annu Rev Biochem 54:665-697, 1985

(*2*) VOSBERG HP: DNA topoisomerases: Enzymes that control DNA conformation. Curr Top Microbiol Immunol 114:19-102, 1985

(*3*) LIU LF: DNA topoisomerase poisons as antitumor drugs. Annu Rev Biochem 58:351-375, 1989

(*4*) ROSS WE: DNA topoisomerases as targets for cancer therapy. Biochem Pharmacol 34:4191-4195, 1985

(*5*) ROSS WE, SULLIVAN DM, CHOW KC: Altered function of DNA topoisomerases as a basis for antineoplastic drug action. *In* Important Advances in Oncology (DeVita VT Jr, Hellman S, Rosenberg SA, eds). Philadelphia: Lippincott, 1988, pp 65-81

(*6*) HSIANG YH, LIU LF: Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. Cancer Res 48:1722-1726, 1988

(*7*) TEWEY KM, ROWE TC, YANG L, ET AL: Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science 226:466-468, 1984

(*8*) NELSON EM, TEWEY KM, LIU LF: Mechanism of antitumor drug action: Poisoning of mammalian DNA topoisomerase II on DNA by 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide. Proc Natl Acad Sci USA 81:1361-1365, 1984

(*9*) ANDOH T, ISHII K, SUZUKI Y, ET AL: Characterization of a mammalian mutant with a camptothecin-resistant DNA topoisomerase I. Proc Natl Acad Sci USA 84:5565-5569, 1987

(*10*) GUPTA RS, GUPTA R, ENG B, ET AL: Camptothecin-resistant mutants of Chinese hamster ovary cells containing a resistant form of topoisomerase I. Cancer Res 48:6404-6410, 1988

(*11*) GUPTA R: Genetic, biochemical and cross-resistance studies with mutants of Chinese hamster ovary cells resistant to the anticancer drugs, VM-26 and VP-16-213. Cancer Res 43:1568-1574, 1983

(*12*) BERAN M, ANDERSSON BS: Development and characterization of a human myelogenous leukemia cell line resistant to 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide. Cancer Res 47:1897-1904, 1987

(*13*) DANKS MK, YALOWICH JC, BECK WT: Atypical multiple drug resistance in a human leukemic cell line selected for resistance to teniposide (VM-26). Cancer Res 47:1297-1301, 1987

(*14*) GLISSON B, GUPTA R, SMALLWOOD-KENTRO S, ET AL: Characterization of acquired epipodophyllotoxin resistance in a Chinese hamster ovary cell line: Loss of drug-stimulated DNA cleavage activity. Cancer Res 46:1934-1938, 1986

(*15*) ZWELLING LA, HINDS M, CHAN D, ET AL: Characterization of an amsacrine-resistant line of human leukemia cells. Evidence for a drug-resistant form of topoisomerase II. J Biol Chem. In press

(*16*) DANKS MK, SCHMIDT CA, CIRTAIN MC, ET AL: Altered catalytic activity of and DNA cleavage by DNA topoisomerase II from human leukemic cells selected for resistance to VM-26. Biochemistry 27:8861-8869, 1988

(*17*) DANKS MK, SCHMIDT CA, DENEKA DA, ET AL: Increased ATP requirement for activity of and complex formation by DNA topoisomerase II from human leukemia CCRF-CEM cells selected for resistance to teniposide. Cancer Commun 1:101-109, 1989

(*18*) DRAKE FH, ZIMMERMAN JP, MCCABE FL, ET AL: Purification of topoisomerase II from amsacrine-resistant P388 leukemia cells. Evidence for two forms of the enzyme. J Biol Chem 262:16739-16747, 1987

(*19*) SULLIVAN DM, LATHAM MD, ROWE TC, ET AL: Purification and characterization of an altered topoisomerase II from a drug-resistant Chinese hamster ovary cell line. Biochemistry 28:5680-5687, 1989

(*20*) TAN KB, MATTERN MR, ENG WK, ET AL: Nonproductive rearrangement of DNA topoisomerase gene is correlated with resistance to topoisomerase inhibitors. J Natl Cancer Inst 81:1732-1735, 1989

(21) BECK WT, CIRTAIN MC, DANKS MK, ET AL: Pharmacological, molecular, and cytogenetic analysis of "atypical" multidrug-resistant human leukemic cells. Cancer Res 47:5455-5460, 1987
(22) PER SR, MATTERN MR, MIRABELLI CK, ET AL: Characterization of a subline of P388 leukemia resistant to amsacrine: Evidence of altered topoisomerase II function. Mol Pharmacol 32:17-25, 1987
(23) OSHEROFF N: Eukaryotic topoisomerase II. Characterization of enzyme turnover. J Biol Chem 261:9944-9950, 1986
(24) FERNANDES DJ, DANKS MK, BECK WT: Decreased nuclear matrix DNA topoisomerase II (topo II) in VM-26 resistant human leukemia cells. Proc Am Assoc Cancer Res 30:502, 1989
(25) REMY JJ, BELEHRADEK J, JACQUEMIN-SABLON A: Expression of drug sensitivity and tumorigenicity in intraspecies hybrids between 9-hydroxyellipticine-sensitive and -resistant cells. Cancer Res 44:4587-4593, 1984
(26) WOLVERTON JS, DANKS MK, SCHMIDT CA, ET AL: Genetic characterization of the multidrug-resistant phenotype of VM-26-resistant human leukemic cells. Cancer Res 49:2422-2466, 1989
(27) JONES PA, TAYLOR SM: Cellular differentiation, cytidine analogs and DNA methylation. Cell 20:85-93, 1980
(28) NITISS J, WANG JC: DNA topoisomerase-targeting antitumor drugs can be studied in yeast. Proc Natl Acad Sci USA 85:7501-7505, 1988
(29) UEMURA T, YANAGIDA M: Isolation of type I and II DNA topoisomerase mutants from fission yeast: Single and double mutants show different phenotypes in cell growth and chromatin organization. EMBO J 3:1737-1744, 1984
(30) DAVIES SM, HARRIS AL, HICKSON ID: Overproduction of topoisomerase II in an ataxia telangiectasia fibroblast cell line: Comparison with a topoisomerase II-overproducing hamster cell mutant. Nucleic Acids Res 17:1337-1351, 1989
(31) DAVIES SM, ROBSON CN, DAVIES SL, ET AL: Nuclear topoisomerase II levels correlate with the sensitivity of mammalian cells to intercalating agents and epipodophyllotoxins. J Biol Chem 263:17724-17729, 1988
(32) TAN KB, MATTERN MR, BOYCE RA, ET AL: Elevated DNA topoisomerase II activity in nitrogen mustard-resistant human cells. Proc Natl Acad Sci USA 84:7668-7671, 1987
(33) ENDICOTT JA, LING V: The biochemistry of P-glycoprotein-mediated multidrug resistance. Annu Rev Biochem 58:137-171, 1989
(34) VAN DER BLIEK AM, BORST P: Multidrug resistance. Adv Cancer Res 52:165-203, 1989