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Review

DNA topoisomerase I and II in cancer chemotherapy: update and perspectives

Yves Pommier

Laboratory of Molecular Pharmacology, Division of Cancer Treatment, National Cancer Institute, BLDG 37, RM 5C27, National Institutes of Health, Bethesda, MD 20892, USA

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Introduction

The extreme length of DNA molecules as well as the structural attachment of the DNA to a structural matrix in the nuclei of eukaryotic cells are responsible for the topological constraints associated with strand separation of the Watson-Crick double-helix, which takes place during DNA metabolism (replication, transcription, repair recombination, mitosis ...). DNA topoisomerases are nuclear enzymes that modulate the topological structure of DNA by making transient DNA breaks. There are two types of mammalian DNA topoisomerases: topoisomerases I and II [57, 93, 94]. Whereas DNA topoisomerase II inhibitors are well established as anticancer agents [57, 67], topoisomerase I inhibitors are presently undergoing phase I trial (for review see [67, 74]).

DNA topoisomerase II

Eukaryotic DNA topoisomerase II has been identified as the target of a number of potent anticancer drugs, including anthracyclines, amsacrines, ellipticines, anthracenediones (DNA intercalators), and nonintercalating etoposides (VP-16, VM-26) [57, 67]. Its bacterial counterpart, DNA gyrase, is the target of quinolone antibiotics (for review see [36]). DNA topoisomerase II is associated with the nuclear matrix of interphase cells and is a major constituent of the chromosome scaffold [2, 23, 28]. The latter is consistent with the enzyme's essential role in chromosome condensation [64, 78, 96] and separation of daughter DNA molecules at the end of DNA replication (decatenation) [19, 34, 89]. Topoisomerase II, along with topoisomerase I, is also a DNA-relaxing enzyme, which removes DNA supercoiling and torsional tension as they arise during transcription and DNA replication [5, 6, 53, 97].

Perspectives: molecular interactions and rational drug design

DNA topoisomerase II can be purified from a variety of tumors [20, 59, 60, 69] and normal tissues (calf thymus [31], avian erythrocytes [63], *Drosophila* cells [41, 66]). Two forms of the enzyme have been identified: topoisomerases II alpha and beta (mol. wt., 170 and 180 kDa, respectively) [21]. The purified enzymes can be used to test the effects of inhibitors with purified DNA. Topoisomerase II inhibitors poison the enzyme by trapping DNA-strand passage intermediates that can be detected as "cleavable (or cleavage) complexes" [86]. These cleavable complexes are protein-linked DNA single- and double-strand breaks that are covalently linked to the enzyme by a phosphotyrosine residue at their 5′-DNA termini. Topoisomerase II inhibitors from different classes (anthracyclines, acridines, ellipticines, anthracenediones, epipodophyllotoxins) trap cleavable complexes at different DNA sites [10, 70, 72, 86]. Base-sequence analysis of a large set of such sites reveals that the base-sequence selectivity of different drugs corresponds to sites having a specific base in the immediate flank of the cleavage site. The preferred base is either at the 5′-terminus – adenine (A) for acridines [73] – or at the 3′-DNA terminus – A for anthracyclines [9], cytosine (C) for etoposides [73], and thymine (T) for ellipticines [25]. Our current interpretation of this observation is that the drugs trap the enzyme-DNA complex by binding inside the cleavage sites and stacking with the preferred base. Further investigation of the "drug-stacking model" may await the resolution of the enzyme-drug-DNA ternary complexes by either crystallography or nuclear magnetic resonance (NMR). In addition, drug-resistant topoisomerases II [7, 33, 42] may be useful in defining the enzyme domains involved in drug action.

Purified topoisomerase II assays are currently used in several pharmaceutical companies to screen for new inhib-

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Correspondence to: Y. Pommier

itors. In parallel, structure-activity studies of existing drugs are used for designing more specific and less toxic inhibitors. In the case of etoposides, the sugar is not required for inhibition of purified topoisomerase II [12, 58, 87], whereas the presence of a hydroxyl residue on the pendant benzene ring is critical [80, 84]. Similarly, the anthracycline sugar is not required for topoisomerase II inhibition since doxorubicinone (the doxorubicin aglycone) is active against purified topoisomerase II (Pommier et al., unpublished results). Since the sugar is important for stabilizing DNA binding, one may expect that potent anthracycline derivatives with lower DNA-binding activity can be synthesized. This may be useful for eliminating drug side effects that may be related to DNA binding (genotoxicity). In this respect, it is noteworthy that 4-demethoxy-daunorubicin (Idarubicin) has a lower DNA-binding affinity but is more potent both as a topoisomerase II inhibitor and as an antitumor agent than daunorubicin [10]. The daunosamine sugar may also be involved in the anthracycline cardiotoxicity and in the reduced activity of the existing anthracyclines against multidrug-resistant cells. The charged amine at the 3′-position may interact both with cardiolipin and with P-glycoprotein. Hence, deaminated anthracyclines have been synthesized and shown to be equipotent to their parent compounds in sensitive cell lines while being markedly more active in multidrug-resistant cells.

Perspectives: determinants of sensitivity and resistance

There are at least two well-defined mechanisms of resistance to topoisomerase II inhibitors: (1) multidrug resistance involving plasma membrane P-glycoprotein (PgP) and (2) topoisomerase II alterations (reduction of enzyme levels/drug-resistant enzyme). In doxorubicin-resistant cell lines, these two alterations are usually associated [26, 83], probably because doxorubicin (as well as VP-16) is a substrate for PgP. Since some other drugs such as 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide (m-AMSA) are poor substrates for PgP while being potent topoisomerase II inhibitions, the design of new analogs with improved activity against multidrug resistance seems possible.

It is now possible to assay DNA topoisomerases I and II and their transcripts in tumor samples using specific antibodies [23, 38, 69, 82] and genetic probes [15, 17, 49, 88]. Since there is good correlation between the enzyme content and the activity of topoisomerase inhibitors in cultured cells [52], it should be interesting to correlate enzyme activity and therapeutic response to topoisomerase inhibitors in human tumors.

It is well established that topoisomerase II inhibitors differ from each other in their spectrum of activity in different tumors. The finding that each class of drug produces cleavable complexes in different regions of the genome suggests a rationale for testing whether differential sensitivity is due to specific gene-damage differences. For instance, we have recently found that m-AMSA induces preferential cleavage in the *c-myc* gene promoter. This may explain why cell lines that overexpress *c-myc*, such as promyelocytic HL-60 cells, are hypersensitive to m-AMSA [77, 99]. One may expect that other oncogenes may be specifically sensitive to other topoisomerase II inhibitors. If this hypothesis is correct, analysis of the oncogenic profile of a given tumor may provide some clues as to which toposiomerase II inhibitor should be used. The goal of these different approaches is the tailoring of cancer chemotherapy for individual patients so as to improve the overall success rate.

Other determinants of sensitivity and resistance remain to be explored since both normal and tumor cells have topoisomerase II. Cell killing is a multistep process in which topoisomerase II inhibition is necessary but is clearly not sufficient. Experimental evidence indicates that protein and RNA synthesis as well as intracellular calcium levels are important for cell killing [3, 14, 18, 81]. Moreover, induction of DNA recombinations by drug-induced DNA breaks may play an important role in cell killing [1, 68, 71]. DNA repair, cell-cycle regulation, and programmed cell death (apoptosis) may be altered in tumor cells [4, 16, 51].

DNA topoisomerase I

Camptothecin was isolated by Wall and co-workers 20 years ago as the active alkaloid of extracts from the Chinese tree *Camptotheca acuminata* [90, 91]. Early clinical trials with the sodium salt of camptothecin in the 1970s were discontinued because of severe toxicity [29, 61]. Almost 10 years ago, camptothecin was found to inhibit selectively eukaryotic DNA topoisomerase I [37]. This finding plus the discovery that the active form of the drug was not its sodium salt but rather the lactone [45] led to the development of new water-soluble derivatives (topotecan [48] and CPT-11 [56]). In parallel, camptothecin was found to be extremely active in xenograft models [30].

DNA toposiomerase I relaxes DNA supercoiling by making transient DNA single-strand breaks [11, 94, 95]. It is concentrated in nucleoli where ribosomal RNA (rRNA) is made [24, 27, 62]. DNA topoisomerase I is expressed continuously during the cell cycle and in quiescent cells, whereas topoisomerase II expression increases during the S phase of the cell cycle and is almost absent in quiescent cells [22, 32]. Thus, DNA topoisomerase I can be targeted in slowly growing tumors that are currently resistant to chemotherapy. Recent evidence suggests that DNA topoisomerase I is also essential for the genomic insertion of viral DNA [8, 92]. This suggests that topoisomerase inhibitors may be useful as antiviral agents [55, 76].

Perspectives: clinical trials of camptothecin derivatives

Two water-soluble derivatives are currently undergoing phase I/II clinical trials: topotecan and CPT-11. Topothecan (hycamptamine) is 9-(dimethylamino)methyl-10-hydroxycamptothecin [48]. Water solubility is conferred by the charged amino group on the 9-substituent. However, the same charge may be responsible for the possible cross-resistance of multidrug-resistant cell lines to the drug [13]. CPT-11, which is 7-ethyl-10-(4-[1-piperidino]-1-piperid-

ino)carbonyloxycamptothecin, is only a weak topoisomerase I inhibitor [56]. The active form of the drug is its metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) [50]. Other camptothecin derivatives are being tested. Indeed, structure-activity studies have demonstrated that substitutions on the A-ring at positions 9, 10, and 11 increase drug potency [39, 45, 74]. 9-Aminocamptothecin is being developed by the NCI, and 10,11-methylenedioxycamptothecin is at least 10 times more potent than camptothecin [65]. Molecular modeling studies may be useful to synthesize new drug molecules derived from the camptothecin structure.

Perspectives: identification of the camptothecin-binding site

Two observations strongly suggest that camptothecin binds to a stereospecific site on topoisomerase I-DNA complexes. First, structure-activity data show that only the 20-S form (natural camptothecin) is active, whereas its 20-R stereoisomer is inactive [45]. Second, we and other investigators have shown that camptothecin traps topoisomerase I-cleavable complexes at sites that have a G at their 5′-DNA terminus [44, 46, 47, 75], indicating a sensitivity for the base immediately adjacent to the cleavage site. This led us to postulate that camptothecin inhibits topoisomerase I by stacking along the 5′-terminus G and forming a stable ternary complex with the enzyme and the DNA [46]. This model is comparable with that described above for topoisomerase II inhibitors. Its testing is awaiting crystallography and/or NMR studies. As in the case of topoisomerase II, purified topoisomerase I is currently used for screening new inhibitors and for rational drug design.

Perspectives: determinants of sensitivity and resistance

The availability of topoisomerase I antibodies [43, 82, 98] and genetic probes [17, 49, 98] make it possible to determine topoisomerase I enzyme and transcripts in tumor samples and to investigate the relationship between enzyme levels and drug activity. In the case of a positive correlation, topoisomerase I monitoring may become useful in predicting tumor response to camptothecin in individual patients.

The formation of cleavable complexes is not sufficient for cell killing by camptothecin. There is good evidence that a collision of moving replication forks with camptothecin-stabilized topoisomerase I-cleavable complexes converts the reversible cleavable complexes into lethal complexes [35, 40]. Such complexes probably consist of broken replication forks [40, 79]. This fundamental observation probably explains why short camptothecin treatments are relatively ineffective, since in this case a significant fraction of the tumor cells may be outside the S phase [35]. Other cellular determinants for camptothecin activity remain to be determined. One way to identify them may be to characterize cell lines that are naturally either hypersensitive or resistant to camptothecin. Finally, it is also important that camptothecin-resistant cell lines with topoisomerase I mutations be isolated so as to characterize the camptothecin-binding site [54, 85].

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