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Novel actions of inhibitors of DNA topoisomerase II in drug-resistant tumor cells

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Abstract. We review herein current work on the cytotoxic and cellular actions of two classes of inhibitors of DNA topoisomerase II: one represented by etoposide and teniposide, which stabilize DNA-protein complexes, and another represented by merbarone and aclarubicin, which do not stabilize such complexes. We discuss current concepts of protooncogene activation and cell cycle perturbations by some of these inhibitors and summarize recent findings of novel actions of the latter compounds in tumor cells that express a mutant topoisomerase II.

Key words: Topoisomerase II inhibitors – DNA-protein complexes – Multidrug resistance

Topoisomerases, anticancer drugs, and the altered topo II multidrug-restistance phenotype

The DNA topoisomerases are important enzymes involved in resolving topological constraints in DNA in association with such cellular functions as transcription, translation, and chromatid separation (reviewed in [29, 30, 38, 39]). In mammalian cells, at least two forms of the topoisomerases have been described and their cDNAs, cloned: DNA topoisomerase I (topo I), which encodes a protein of ~100 kDa [19, 25], and DNA topoisomerase II (topo II), which exists as two "isoforms," known as α and β, that encode proteins of ~170 and ~180 kDa, respectively [18, 36, 37]. Clinically, these enzymes are the targets of important oncolytic agents: camptothecin and its derivatives topotecan, 9-amino camptothecin, and CPT-11 specifically inhibit topo I [9], whereas epipodophyllotoxins, anthracyclines, aminoacridines, and anthracinediones have been shown to inhibit topo II [26]. It is thought that some of these topo II inhibitors have a greater specificity for the α rather than the β form of topo II [13], whereas the reverse may be the case for the anthracinedione mitoxantrone [14]. In general, these inhibitors of topo I and II appear to block religation of cleaved DNA and therefore stabilize complexes of DNA and topoisomerase [31]. There are, however, inhibitors of topo II that do not stabilize protein-DNA complexes and appear to exert their action by blocking the binding of the enzyme to the DNA; such drugs are typified by merbarone, aclarubicin, and fostriecin [6, 8, 33].

As with most anticancer agents, tumor cells have developed mechanisms to resist the cytotoxic actions of the topoisomerase inhibitors. In general, cells selected for resistance to the epipodophyllotoxins, anthracyclines, aminoacridines, and anthracinediones express a form of multidrug resistance (MDR) restricted to these types of topo II inhibitors, and the characteristic feature of such cells is the expression of decreased amounts or activities of topo II. We have termed this phenotype "at-MDR", for *m*ulti*d*rug *r*esistance associated with altered topo II [10]. The cellular, pharmacological, and molecular features associated with tumor cells expressing altered DNA topoisomerase II have been well described (reviewed in [2–4, 16, 35]). In the absence of other resistance mechanisms, such cells generally remain sensitive to the cytotoxic actions of antimitotic agents, antimetabolites, and topo I inhibitors such as camptothecin and topotecan. Of considerable interest, these cells also remain relatively sensitive to that class of topo II inhibitors typified by merbarone, SN22995, aclarubicin, and the like, which do not stabilize DNA-topoisomerase complexes [8]. Despite this sensitivity to the non-complex-stabilizing topo II inhibitors, at-MDR is apparently due to expression of either mutant or decreased amounts of topo IIα [7, 15, 41], although there is some evidence suggesting that topo IIβ may play a role in this form of resistance [14]. The issue will not be resolved until

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the mutant genes are transfected into and expressed in cells or until an appropriate "knockout" gene deletion experiment is designed.

Although much is now known about the phenomenon of at-MDR, many questions remain. For example, at the clinical level, we do not yet know whether tumor cells from patients who are refractory to anthracyclines or epipodophyllotoxins express features of the at-MDR phenotype, viz. altered topo II. At a more fundamental level, we do not fully understand the mechanisms by which stabilization of DNA-topoisomerase complexes is translated into a lethal event. Although data have been presented to suggest that the drug-stabilized DNA-topo complexes may physically block movement of the replication fork [17, 40], those experiments do not provide detailed molecular explanations about how the formation of complexes actually causes cell death. In fact, those experiments offer little insight into the mechanism(s) by which signals, possibly provided by the formation of these complexes, are sensed by the cell and translated into a lethal event. Moreover, data suggesting "collision" of the replication fork with DNA-topo complexes do not address the issue of cell death mediated by inhibitors of topo II that do not stabilize DNA-topo complexes, since there would likely be no physical block of the replication fork in those cases.

In addition to these questions, we have but little understanding of the physiological consequences to the cell of the presence of a mutant or altered DNA topoisomerase, especially DNA topo II. We have found that at-MDR cells of increasing resistance to teniposide (VM-26) reach progressively lower plateaus of cell density; moreover, these cells show progressively less tolerance to growth at elevated temperature [3]. These results suggest that the expression of altered (mutant) topo II is not without effect on cellular physiology.

Early signals: what happens after the formation of DNA-topo II complexes?

Although it has been shown that treatment of cells with drugs that stabilize DNA-topo II complexes ultimately results in cell death, the early events preceding and following the stabilization of these complexes have not been detailed. It is known that the stabilization of DNA-topo II complexes by drugs such as VM-26 results in activation of *c-jun* transcription [21, 32]. Our preliminary results revealed that teniposide induction of *c-jun* expression was progressively attenuated in cell lines of increasing at-MDR; of interest, transcription of *c-fos* was not induced under these conditions ([22, 23]; R. Kim and W. T. Beck, manuscript in preparation). Further, the kinetics of *c-jun* mRNA induction paralleled that observed for the formation of DNA-topo II complexes produced by teniposide (R. Kim and W. T. Beck, manuscript in preparation). Thus, our experiments with the resistant cells indicate that there is a relationship between DNA-topo II complex formation and induction of *c-jun* transcription. It is likely that this activation of *c-jun* is a general response to DNA damage and that the specificity in the signalling of a cytotoxic event resides elsewhere.

Later signals: what happens after induction of *c-jun*?

Cell death in response to certain anticancer drugs and ionizing radiation represents the culmination of a complex set of biochemical and molecular events. In many instances, activation of a program of cell death, or apoptosis, is observed in response to these stimuli [5, 28, 32, 34], and it has been suggested that activation of protooncogenes such as *c-jun* is one of the early events involved in initiating this pathway [28, 32]. To the best of our knowledge, no direct mechanistic connection between protooncogene activation and subsequent cell death has been described, and this represents a primary research challenge. However, using our drug-resistant cell lines, we found a dose- and time-dependent relationship between these early events and subsequent apoptosis. For example, in work to be presented elsewhere, we have found that DNA in CEM cells is fragmented into characteristic internucleosomal "ladders" of 180–200 bp after treatment with VM-26, and the time of appearance of these ladders suggests that they are produced after activation of *c-jun* transcription; in contrast to the CEM cells, the appearance of these ladders is delayed or decreased in CEM cell lines of increasing resistance to VM-26 ([22, 23]; R. Kim and W. T. Beck, manuscript in preparation). Thus, there is a relationship between early protooncogene activation and subsequent cell death induced by drugs such as VM-26 [28, 32].

We know little about the events occurring between the formation of DNA-protein complexes and the production of apoptotic nucleosomal DNA ladders, but recent results suggest that p53 and other proteins that monitor cell cycle "checkpoints" may be involved [12, 20]. Topo II inhibitors have been shown to activate p53, leading to G1 arrest [12, 20]. These cells subsequently traverse the cell cycle, and if their DNA has become fragmented due to activation of Ca^{+2}-dependent endonucleases or altered intracellular pH, they go on to die through an apoptotic mechanism [1, 5, 20, 34]. Thus, inhibition of topo II by drugs may be only an initial action of these agents; rather, the major effect of inhibition of topo II and other targets may be to interfere with the cell cycle machinery. For example, Kung and colleagues [24] have recently shown that the differences between human and hamster cells in response to inhibition of DNA synthesis reflects differences in response to perturbations in the cell cycle. Furthermore, results from Lowe et al. [27] also suggest that the cytotoxic actions of several anticancer agents is related to the p53 status of the cells. These and other findings suggest that cytotoxic agents may exert their actions by indirectly interfering with the cell cycle and perhaps overriding cell cycle checkpoints. (If this concept is correct, a corollary would be that in at-MDR cells, either p53 may not be activated following cytotoxic drug administration or a mutant p53 may be activated.) Thus, the connection between the formation of DNA-topo II complexes, transcription of *c-jun*, and activation of programmed cell-death pathways is proving to be quite complicated. A general framework for this concept is presented in Fig. 1.

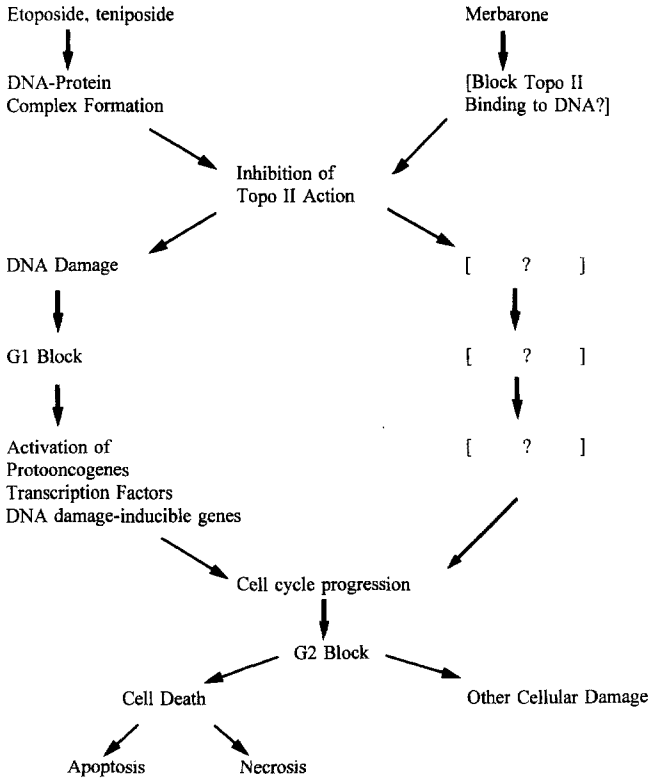

Fig. 1. Possible cellular perturbations leading to cell death following treatment with two classes of inhibitors of DNA topoisomerase II

Unusual effects of non-complex-stabilizing inhibitors of DNA topo II in at-MDR cells

As "classic," complex-stabilizing topo II inhibitors produce the aforementioned effects, we asked whether all topo II inhibitors would cause similar actions. We examined a series of drugs known to inhibit topo II without stabilizing DNA-protein complexes; these drugs included merbarone, fostriecin, aclarubicin, RP60475F (intoplicine), and SN22995. We and other investigators have presented data suggesting that these agents may inhibit topo II by blocking its binding to DNA [6, 8, 33]. In contrast to etoposide, merbarone may cause general DNA degradation as well as nucleosomal ladders in wild-type and at-MDR CEM cells, but this effect is not seen until 48–72 h after drug treatment (R. Kim and W. T. Beck, unpublished results). Unexpectedly, we found that our at-MDR cells expressed little, if any, cross-resistance to these non-complex-stabilizing drugs; i.e., the wild-type and at-MDR cells were approximately equally sensitive to the growth-inhibitory effects of these agents [8]. However, when we examined the cell cycle distribution of CEM and at-MDR cells treated with merbarone or SN22995, we found striking differences in the way wild-type and at-MDR cells responded: whereas CEM cells remained arrested in the G2/M phase throughout the 72-h period of the experiment, at-MDR cells, which were initially blocked in the G2/M phase, broke through this block and re-replicated their DNA; this occurred without further cell division. A summary of these findings is presented in Fig. 2. When we examined the chromosomes from these merbarone- and SN22995-treated cells, we found that whereas those from CEM cells were either normal or fragmented, those from CEM/VM-1 and CEM/VM-1–5 cells were elongated and intertwined [8].

What can account for the disparate results obtained between wild-type and at-MDR cells? We offer the following hypothesis: the at-MDR cells harbor two point mutations in the topo IIα gene [7, 11]. Furthermore, Bugg et al. [7] have reported that whereas only the wild-type topo IIα allele is expressed in CEM cells, both the wild-type and the mutant alleles are expressed in at-MDR cells. Accordingly, we propose that drugs such as merbarone and SN22995 may specifically inhibit the action of the wild-type enzyme, having less effect on the mutant enzyme. This could cause the cells to re-replicate their DNA and also to permit the mutant enzyme to exert its action; in this case, since one of the functions of topo II is to separate chromosomes at mitosis, we suggest that the mutant enzyme either is not capable of doing this or, if it can, it does not do so as efficiently as the wild-type enzyme.

Future directions

DNA-protein complex-stabilizing drugs increase the expression of *c-jun* mRNA in CEM and other cells, and this

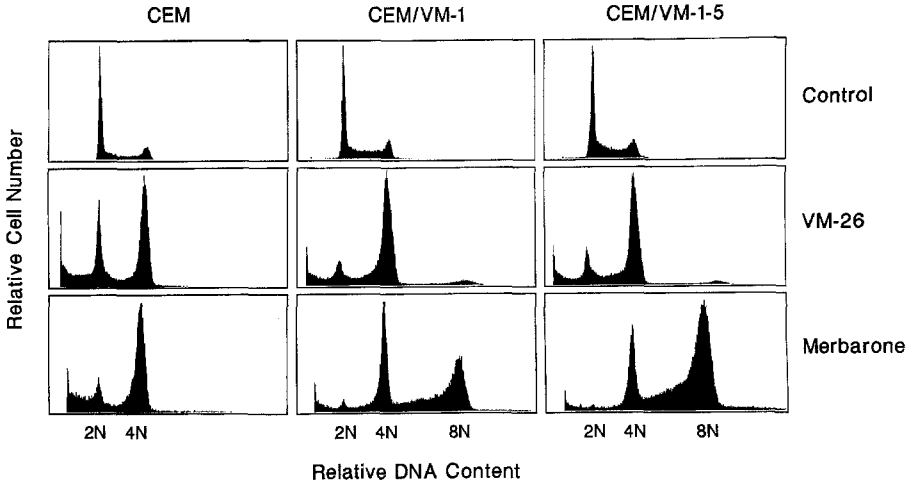

Fig. 2. Comparison of effects of teniposide (VM-26) and merbarone on the cell cycle in CEM, CEM/VM-1, and CEM/VM-1-5 cells. Cells were treated for 72 h with equitoxic concentrations of the respective drugs (see [8] for experimental conditions). Data taken from Chen and Beck [8], with permission of the American Association for Cancer Research, Inc.

expression is progressively attenuated in cell lines of increasing resistance to VM-26. The induction of *c-jun* transcription appears to be related to and possibly, a consequence of the formation of DNA-protein complexes. However, results obtained with "nonclassic" inhibitors of topo II suggest that DNA-protein complex formation is not an essential requirement to mediate cell death. Finally, the idea that inhibition of DNA topo II through induction of DNA strand breaks is a primary cause of cell death requires reevaluation in light of current knowledge of cell cycle biochemistry and checkpoint proteins. Examination of the effects of "nonclassic" topo II inhibitors on *c-jun* induction is currently in progress, as is investigation of the differences between wild-type and at-MDR cells in their expression of proteins involved in cell cycle regulation and drug responsiveness.

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