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Antitopoisomerase Drug Action and Resistance

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INTRODUCTION

THROUGH THE years we have come to understand that anticancer drug resistance has different biochemical and molecular mechanisms depending on the class of drug. Tumour cell insensitivity to "natural product" anticancer drugs frequently appears as multidrug resistance (MDR) that can take several forms associated with different proteins: overexpression of P-glycoprotein [1, 2], overexpression of the multidrug resistance-associated protein, MRP [3, 4], overexpression of LRP [5, and references therein], recently shown to be a major vault protein [6], and alterations in the essential nuclear enzyme, DNA topoisomerase II [7, 8]. There will doubtless be other forms of MDR associated with other drug classes. Finally, MDR arising from alterations in cellular physiology that alter or abrogate cell killing pathways is becoming a major new arena in the study of anticancer drug resistance [9, 10, and references therein].

The focus of this paper is a discussion of the DNA topoisomerases, especially the type II enzymes, and their roles in chemotherapy and in drug resistance. We will review the actions of topoisomerases in cell physiology, discuss biochemical mechanisms of several classes of topoisomerase inhibitors, and examine current concepts of tumour cell resistance to these agents. Drug actions and resistance mechanisms will also be discussed in the context of pathways associated with cell killing.

DNA TOPOISOMERASES AND THEIR ANTITUMOUR INHIBITORS

DNA topoisomerases are nuclear enzymes that play essential roles in DNA replication, transcription, chromosome segregation, and DNA recombination (reviewed in [11–14]). All cells have two major forms of topoisomerases: the type I enzymes, that make single-stranded cuts in DNA, and the type II enzymes, that cut and pass double-stranded DNA [11–14]. There have been several newly discovered topoisomerases in both prokaryotic and eukaryotic cells. For example, yeast cells have an additional type I nuclear topoisomerase that is homologous to the bacterial type I enzyme but not to other eukaryotic topoisomerases [15], and a recent report has detected a gene for a similar protein in human cells [16]. Moreover, yeast cells have an additional protein, termed HPR1, with significant homology to other eukaryotic type I topoisomerases [17]. However, topoisomerase activity with the purified protein has not yet been demonstrated. In mammalian cells, there are two isozymes of topoisomerase II, a 170 kd form termed p170, or α, and a 180 kd form termed p180, or β [7, 8, 18]. These two proteins are the products of different genes, located in human cells on chromosomes 17q21–23 [19] and 3q [20], respectively. These isoforms are expressed differently through the cell cycle: topoisomerase II α is preferentially expressed in proliferating cells during S phase [21], whereas topoisomerase IIβ appears to be expressed at all points in the cell cycle, with no appreciable differences between proliferating and non-proliferating cells [22]. Some data have indicated that the β isoform is preferentially localised to the nucleolus [23], leading to the suggestion that it may play an important role in transcription, but there is no direct evidence to support this hypothesis.

Topoisomerases have been shown to be targets for clinically important antitumour agents [24, 25]. For example, topoisomerase I is a very specific target of camptothecin and its newer analogues such as topotecan, 9-amino-camptothecin, and irinotecan (CPT-11) [26]. Recent phase I/II studies have demonstrated that topotecan and irinotecan may be quite effective in the treatment of a variety of solid tumours including breast carcinoma and small cell lung cancer [27, 28] (see below). A wide range of agents have been identified that specifically inhibit topoisomerase II, including the epipodophyllotoxins (etoposide and teniposide), aminoacridines (e.g. amsacrine), and some anthracyclines (e.g. doxorubicin, daunorubicin) [24, 25], as well as some novel agents such as merbarone [29] and the bisdioxopiperazines (e.g. ICRF-187) [30]), which will be discussed below.

Catalytic mechanisms of DNA topoisomerase inhibition

Inhibitors of DNA topoisomerases appear to be capable of interfering with enzyme catalysis at several distinct points of the enzyme reaction. This was clear from studies of the inhibitors of bacterial type II topoisomerase (DNA gyrase). Coumarin antibiotics and related compounds inhibit the ATPase activity of the enzyme [31, 32], while quinolones interfere with the breakage/reunion reaction of gyrase [33, 34]. Progress on the solution of the three-dimensional structure of

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the enzyme will likely lead to additional insights into enzyme mechanism, futher illuminating the mechanisms of drug action.

To understand the basis by which most inhibitors of the topoisomerases exert their actions, it is necessary to understand the catalytic cycle of breakage–reunion of the topoisomerases themselves. Recent biochemical studies have led to a more detailed model for how eukaryotic topoisomerase II accomplishes its reactions. The catalytic cycle of topoisomerase II has been shown to involve several discrete steps, including: recognition/binding, cleavage, strand passage, religation, and enzyme turnover [35], as outlined in Figure 1. The cleavage reaction of topoisomerase II is carried out by the formation of a transient covalent bond between a tyrosine residue of topoisomerase II and the 5′ phosphate of the DNA molecule [35]. The ATPase activity of the enzyme is required for enzyme turnover, but is not required for any of the other steps of the reaction. There is less information concerning the steps in the catalytic cycle of topoisomerase I. Like topoisomerase II, topoisomerase I forms a covalent linkage with DNA, but a tyrosine of the enzyme forms a phosphotyrosine linkage with the 3′ phosphate of the DNA chain [36]. The steps involved in strand passage by topoisomerase I are also poorly understood. One proposed mechanism is that following breakage of the phosphodiester backbone, the broken DNA strand can freely swivel, thereby changing the linking of the DNA molecule by one or many steps [7]. Alternately, topoisomerase I may carry out directed strand passage in a manner analogous to the action of topoisomerase II [37, 38].

The antitumour importance of the catalytic cycle of topoisomerases relates to the fact that the clinically useful drugs discussed above—etoposide, doxorubicin, amsacrine, topotecan, etc.—appear to be able to increase the amount of a species that includes the enzyme covalently attached to DNA, thereby turning the enzyme into a cellular poison, leaving the DNA strands broken [24, 25, 35]. The drugs accomplish this by blocking the religation of the cleaved DNA, or by increasing the rate of cleavage of DNA without inhibiting religation [25]. For both of these biochemical mechanisms, the drugs stabilise a "cleaved complex" of DNA and topoisomerase, as shown in Figure 1, the levels of which can be readily measured in the laboratory. The consequences of formation of a covalent complex with DNA include interference with nucleic acid metabolism, the induction of genetic changes due to the presence of DNA strand breaks, and may include the initiation of a programmed cell death (PCD) pathway, as will be discussed below.

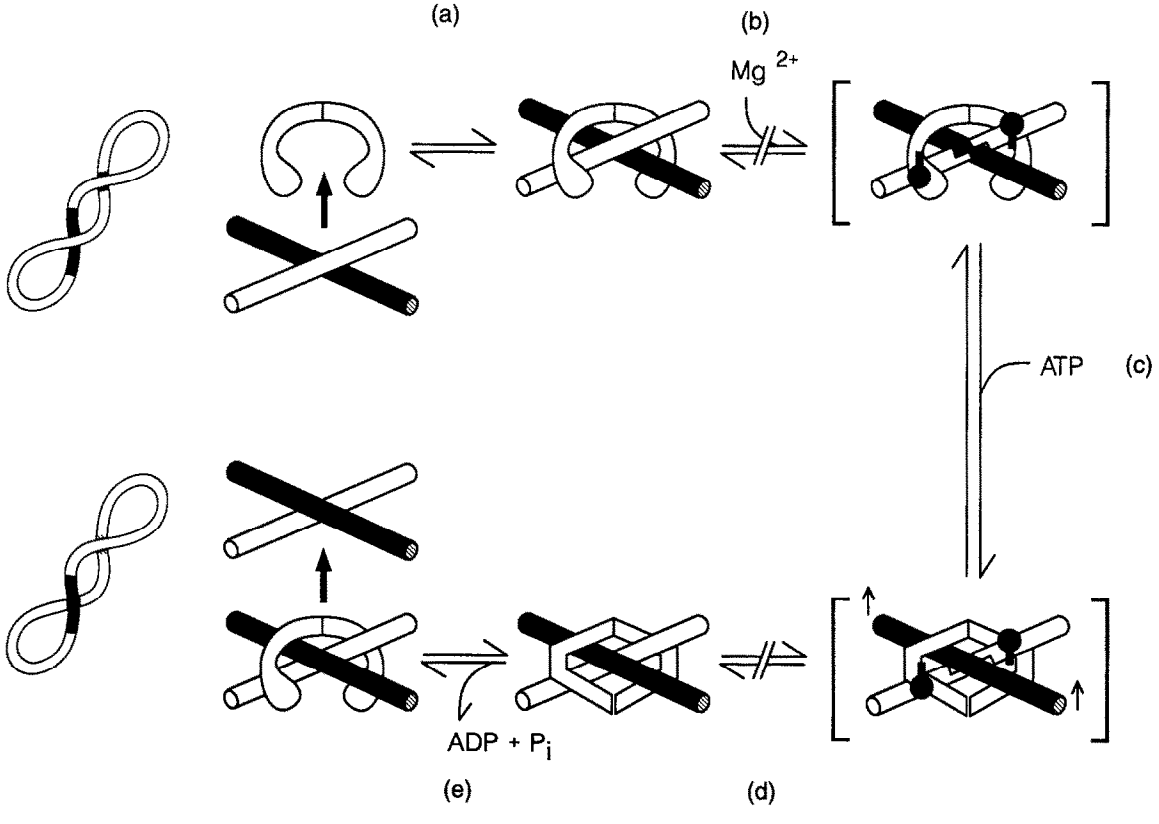

Figure 1. Catalytic cycle of topoisomerase II. The catalytic cycle of DNA topoisomerase II has been dissected by breaking down the overall reaction into a series of discrete steps. (a) The enzyme binds to double-stranded DNA, and may recognise specific features of the DNA structure such as crossed double strands [113]. (b) If Mg^{2+} ion is present, the enzyme can cleave DNA, forming a covalent complex via a phosphotyrosine linkage. Presumably, antitopoisomerase drugs must be present at least at this step in the reaction to produce an effect. In many cases, the drugs may need to be present prior to DNA cleavage. (c) Upon ATP binding the enzyme undergoes a conformational change [114], and DNA strand passage can occur. (d) Following strand passage, the enzyme reverses the cleavage reaction, and utilises the energy of the phosphotyrosine bonds to restore the phosphodiester linkages in both DNA strands. Drugs such as etoposide or amsacrine block the religation of the broken DNA strands. Note that many other antitopoisomerase II drugs do not inhibit religation [115]. For other antitopoisomerase II drugs, such as fluoroquinolones, the drugs increase the apparent forward rate of cleavage [115]. (e) After religation of the strand breaks, ATP hydrolysis occurs; the ATP hydrolysis is coupled with a conformational change that restores the enzyme to a conformation that allows another reaction cycle to proceed. The figure shown was adapted from Osheroff and colleagues [115].

Novel inhibitors of topoisomerase II, typified by drugs such as merbarone, aclarubicin, fostreicin, and bis(2,4,dioxopiperazine) derivatives (e.g. ICRF193, ICRF187) do not appear to stabilise such complexes [29, 30, 40]; rather, they prevent the binding of the enzyme to DNA [41], or block some other step in the topoisomerase II catalytic cycle. However, neither aclarubicin nor fostreicin are specific for topoisomerase II; fostreicin inhibits protein phosphatases [42] while the strong intercalation of aclarubicin may interfere with many different DNA binding proteins [43]. Recent studies in yeast have suggested that bis(2,4,dioxopiperazine) derivatives are highly specific for topoisomerase II [44]. One intriguing mechanism by which these drugs inhibit the enzyme may involve the stabilisation of the binary complex of topoisomerase II and drug in a "closed clamp" configuration [45], thereby blocking the catalytic cycle of topoisomerase II. It is of interest that tumour cells selected for resistance to complex-stabilising inhibitors of topoisomerase II may remain sensitive (or even become hypersensitive) to the catalytic inhibitors [40].

As described above, inhibitors of topoisomerase I, typified by camptothecin and its derivatives, also appear to block the religation step of the topoisomerase I reaction [46]. While many agents appear to inhibit the catalytic activity of topoisomerase I, most bind to DNA very tightly, and have little specificity for topoisomerase I (e.g. berenyl and ethidium bromide) [47]. However, drugs that bind in the minor groove of DNA may be more specific inhibitors of topoisomerase I, although many minor groove binding drugs also stabilise a covalent complex between DNA and topoisomerase I.

From covalent complex to irreversible lesion

How and why does each of these mechanisms of inhibition lead to cell death? The initial clues arose from studies of the inhibitors of bacterial topoisomerase II. Kreuzer and Cozzarelli [50] demonstrated that cell killing by quinolones, which stabilise covalent complexes between bacterial topoisomerase II and DNA, differs dramatically from cell killing that either arises from catalytic inhibitors of bacterial topoisomerase II or from conditional mutations of the enzyme. Studies in model eukaryotic systems such as yeast led to the same conclusion [51]. Subsequent work from many laboratories [52, and references therein] has suggested that the replication fork can collide with the topoisomerase II–DNA complex generating double strand breaks, some of which cannot be reversed by the enzyme. This proposed mechanism provides an explanation of how the covalent complex, which is a reversible lesion, can nonetheless produce lesions that persist after the drug is removed.

The mechanisms described above all lead to the generation of irreversible lesions in DNA. Especially important is the generation of double strand breaks in DNA, since unrepaired double strand breaks are cytotoxic lesions [53]. In addition, the processes that repair double strand breaks are not completely error free [54]. For example, it has been suggested that end-to-end joining of double strand breaks with minimal regard for homology is a major repair pathway for double strand breaks in mammalian cells, but such repair would generate deletions and translocations in the genome [54]. Thus, the loss of genetic information by deletions and translocations may contribute to cell killing by antitopoisomerase agents [55]. In addition, loss of a chromosome end or the generation of dicentric chromosomes can result in further chromosome damage in subsequent cell divisions [53].

The induction of genetic changes by antitopoisomerase agents is an important consideration in their use as anticancer agents. For example, it has been demonstrated that secondary leukaemias, principally AML, can arise following treatment with etoposide [56, 57]. This secondary AML is associated with specific chromosome translocations [58].

What happens when topoisomerase II catalytic activity is inhibited without stabilisation of a covalent complex? A model is suggested from studies with yeast strains carrying temperature-sensitive mutations in *TOP2*, the structural gene for topoisomerase II [59]. Incubation of such yeast cells at the non-permissive temperature results in cytotoxicity as cells proceed through mitosis [60]. Cells lacking topoisomerase II are unable to carry out chromosome condensation or decatenation of sister chromatids prior to mitosis, and attempted segregation of the catenated chromosomes leads to non-disjunction as well as chromosome breakage [61]. Similar phenotypes have been observed by Andoh and colleagues in cells treated with ICRF compounds [62] and by Chen and Beck in "at-MDR" (altered topoisomerase-associated multidrug resistance) cells that harbour mutant topoisomerase II, after treatment with merbarone [40]. Clearly, early steps in the cell killing pathway differ between inhibitors that stabilise covalent complexes and inhibitors that act at other points in the catalytic cycle of topoisomerase II.

CELLULAR CONSEQUENCES OF INTERFERENCE WITH THE ACTIVITY OF DNA TOPOISOMERASES: INITIATION OF PROGRAMMED CELL DEATH PATHWAY(S)

It has been known for some time that DNA damaging agents are able to arrest the progression of cells through the cell cycle [63]. Indeed, cell cycle progression can be arrested at several points that depend either on the proper occurrence of specific landmark events or if conditions are otherwise unfavourable for cell cycle progression [64, 65]. These stopping points are referred to as "checkpoints", and cell cycle arrest at these checkpoints can be a consequence of DNA damage [66]. Thus, checkpoints appear to be critical determinants of a cell's sensitivity to anticancer agents [64, 67]. At a checkpoint, cells are capable of making several choices: stall and wait for alleviation of the deleterious condition; proceed through the cell cycle, despite the unfavourable circumstances; or commit to programmed cell death (PCD). The tumour suppressor protein, p53, is a major factor in this decision-making process at checkpoints. After DNA damage, whether by drugs or ionising radiation, activation of p53 appears to be central in governing a cell's decision to arrest, re-enter the cell cycle, or initiate a PCD response [64, and references therein]. However, while most work has concentrated on p53's role in arrest in G1, p53 has also been shown to play a role in G2 arrest [68, 69], and is important in assessing other landmarks besides the integrity of DNA, e.g. as a checkpoint for spindle integrity [70]. Finally, cell cycle regulatory proteins are also altered following cytotoxic insults. For example, $P21^{WAF1/CIP1/SDI1}$ may be the major inhibitory protein governing progress through the cell cycle [71], and its expression is apparently activated by p53 [72]. Hence, cell cycle arrest following cellular damage is part of a p53-dependent pathway. However, cells that do not express p53 are still capable of undergoing PCD following DNA damage [73],

suggesting that the cytotoxic signal may also be generated independently of p53.

Since apoptosis seems to be critical in cell killing by many agents, the proteins that mediate this effect have been the recent focus of intense study. The first protein that was identified, bcl-2, is able to protect cells from committing to cell death following many different types of cellular damage. The identification of bcl-2 has assisted in the identification of a family of proteins, including bax, a related protein, that can interact with bcl-2 [74]. It has been proposed that the ratios of these proteins serve as a "rheostat" that governs whether or not a cell commits to PCD [75]: more bcl-2 tending towards protection from PCD, more bax tending to push the cell into PCD. Other related proteins have also been identified that may act as a multimeric complex that mediates the decision of whether cells commit to PCD [74]. Bcl-2 has been shown to alter the subcellular distribution of p53, preventing its translocation to the nucleus [76]. This provides one possible mechanism by which bcl-2 might block the apoptotic effects of p53. By contrast, the antipodal partner of bcl-2, bax, is up-regulated by p53 [77]. While we have much to learn about this signalling network, p53 is clearly a central player. Some of the other proteins that act with bcl-2 may play roles in generating and carrying out the death signal arising from DNA damage.

Together, the above results suggest the following pathway of cell killing as related to topoisomerase inhibitors: DNA topoisomerase inhibition→interference with nucleic acid metabolism and conversion to irreversible lesions→cell signaling→PCD pathways (Figure 2). Thus, after damaging DNA by stabilisation of DNA–topoisomerase complexes, topoisomerase inhibitors ultimately may exert their cytotoxic action through initiation of a PCD pathway. DNA damage is unlikely to be the only triggering event by which topoisomerase inhibitors, especially topoisomerase II inhibitors, kill cells. Since the catalytic inhibitors of topoisomerase II do not stabilise these DNA–protein complexes, nor do they cause frank DNA breaks in cells within a few hours after treatment, one can speculate that cytotoxic events mediated by catalytic inhibitors may depend on commitment to cell death at a G2 checkpoint that assesses topoisomerase function. One report suggested that a G2 checkpoint for topoisomerase II action functioned in mammalian cells [78]. However, studies by Andoh and colleagues failed to detect cell cycle arrest with bisdioxopiperazines [79]. Also, yeast topoisomerase II mutants do not exhibit the cell cycle arrest that would be characteristic of a checkpoint of topoisomerase II function [60]. Further studies will be needed to determine whether a G2 arrest is important for cell killing by catalytic inhibitors of topoisomerase II. Clearly, the mechanisms by which tumour cells die in response to a cytotoxic signal involve a complex set of processes with multiple cellular pathways, and our present awareness of how anticancer drugs, especially the DNA topoisomerase inhibitors, may affect these signals is rudimentary.

TUMOUR CELL RESISTANCE TO TOPOISOMERASE INHIBITORS

With the preceding introducion to the topoisomerase inhibitors and their cytotoxic actions, we can now address mechanisms of tumour cell resistance to these agents. Over the last few years, many cell lines have been selected for resistance to inhibitors of either topoisomerase I or topoisomerase II, and these have been described in several reviews [7, 8, 80]. The following sections outline resistance mechanisms that have been identified or suggested, with an emphasis on resistance mechanisms specific for antitopoisomerase agents.

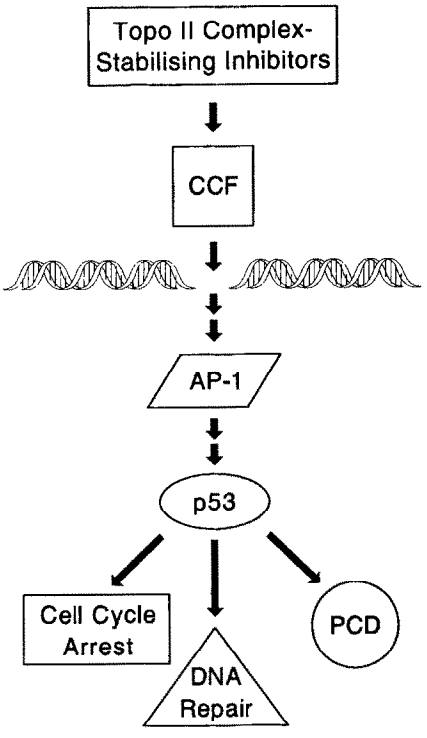

Figure 2. Proposed cytotoxic signalling pathway for complex stabilising inhibitors of mammalian topoisomerase II. Following covalent complex formation (CCF), DNA metabolic complexes result in irreversible breakage of DNA. The irreversible breaks (or perhaps the covalent complexes themselves), can lead to the activation of cell signalling pathways. For example, transcriptional activation of proto-oncogenes such as *FOS* and *JUN* leading to enhanced AP-1 transcriptional activation, and transcriptional activation of the tumour-suppressor gene, *TP53*, is seen. p53 can activate a checkpoint arrest resulting in inhibition of cell cycle traversal. The inhibition of cell cycle progression allows the cell to deal with a potentially mutagenic/cytotoxic insult. The damage may be repaired or, if the DNA damage is too great, the cell may commit to death through activation of a programmed cell death (PCD) pathway involving the *BCL-2/BAX* family of genes.

Decreased drug accumulation

Because some topoisomerase inhibitors (e.g. etoposide, doxorubicin) are substrates for P-glycoprotein (Pgp), cells that express this efflux pump protein will display resistance to these agents due to decreased drug accumulation [1, 2]. Cells expressing Pgp have been shown to be modestly resistant to the camptothecin analogue, topotecan, a likely consequence of this drug being a substrate for Pgp, but other camptothecin analogues are poor substrates for Pgp [81]. Similarly, recent findings indicate that cells selected for resistance to anthracyclines or epipodophyllotoxins can also express MRP, the multidrug resistance-associated protein, and cells expressing the MRP cDNA are resistant to etoposide and doxorubicin [3, 4]. While both Pgp (MDR1) and MRP have been shown to be expressed in the tumours of therapy-resistant patients [82, 83], the importance of these resistance-associated proteins has yet to be fully explored in terms of clinical resistance to inhibitors of topoisomerases.

Drug metabolism

Unique among clinically important topoisomerase inhibitors is CPT-11 (irinotecan), which is prodrug of SN-38, a potent topoisomerase I inhibitor. CPT-11 is converted to SN-38 by a carboxylesterase reaction [84]. While alterations in carboxylesterase activity should affect tumour cell sensitivity to irinotecan, we do not know if the tumour cell expression of carboxylesterases are relevant parameters for tumour cell sensitivity to irinotecan. Alternatively, tumour cells may take up SN-38 produced by carboxylesterase activity from other tissues. Whether metabolism has a major influence on resistance to irinotecan remains to be answered.

Drug resistance by target alteration: non-mammalian systems

Much attention has been paid to target alteration as a mechanism of resistance to antitopoisomerase agents. The model for these studies has in part been the development of quinolone resistance in bacteria [85]. Quinolones are highly specific inhibitors of bacterial topoisomerase II [86], and for high level resistance to occur, the drug sensitive topoisomerase must become drug resistant. As deduced from studies with genetically tractable models, there are two ways that the topoisomerase target can change and generate resistance to complex stabilising drugs: (i) the level of the target enzyme can be reduced, or (ii) the topoisomerase can mutate to a drug resistant form. Thus, these models have permitted the following conclusions (for a more complete discussion of these points see [87]): (1) lower levels of enzyme activity lead to drug resistance; (2) mutations in the structural gene for topoisomerases can lead to high levels of drug resistance; (3) mutations in the topoisomerase genes usually confer recessive drug resistance due to reduced topoisomerase activity; (4) exceptionally, mutations in a topoisomerase gene leading to drug resistance can be partly dominant.

How might a mutation leading to resistance to complex stabilising drugs be dominant? One answer may involve the fact that topoisomerase II is a dimer. If mutant and wild type genes are producing equal amounts of protein, then three species of holoenzymes would be expected: wt:wt, wt:mut and mut:mut in the ratio of 1:2:1. If the wt:mut dimer is drug sensitive, then 3/4 of the topoisomerase in the cell would be drug sensitive, while 1/4 would be drug resistant. By contrast, if the heterodimer is drug resistant, then 3/4 of the enzyme in the cell would be drug resistant and 1/4 would be drug sensitive. If the mut:wt heterodimer is enzymatically inactive, then the mutation would reduce total topoisomerase activity to 1/4 the wild type level. This is probably sufficient to confer significant drug resistance. These considerations suggest that care is needed in interpreting the results of mutations identified in mammalian cell lines selected for resistance to complex stabilising antitopoisomerase II agents. Some of the mutations that are identified may be null or nonsense mutations [88]. These mutations may produce drug resistance by reducing the overall topoisomerase activity in the cell.

Drug resistance by target alterations: mammalian systems

What happens in mammalian cell lines? Resistance to the complex stabilising inhibitors of topoisomerase II is manifest as a type of multidrug resistance, termed "at-MDR", for altered topoisomerase-associated multidrug resistance [7]. Cells selected for resistance to epipodophyllotoxins (e.g. etoposide, teniposide), aminoacridines (e.g. amsacrine), or the "classic" anthracyclines (e.g. doxorubicin, daunorubicin) have frequently been shown to express features of at-MDR (reviewed in [7, 8, 80]). In general, at-MDR cells express crossresistance to complex stabilising inhibitors of topoisomerase II. By contrast, such cells, if they do not express either Pgp or MRP, generally remain sensitive to antimitotic agents, antimetabolites, topoisomerase I inhibitors, and, importantly, catalytic inhibitors of topoisomerase II.

Resistance to topoisomerase I inhibitors follows a similar pattern, except the resistance is specific for these agents [89]. Thus, cells selected for resistance to inhibitors of topoisomerase I are crossresistant to other topoisomerase I inhibitors, and this appears to be a consequence of decreased topoisomerase I activity associated with either decreased expression of the enzyme or mutations altering catalytic activity or drug sensitivity [89].

Decreased activity due to mutation or decreased expression of topoisomerases

In some mammalian cell lines, resistance to the topoisomerase inhibitors is associated with either point mutations in the genes [7, 90–92], or deletions [93]. Summarised in Figure 3 and [94] are the locations of the mutations identified to date in mammalian topoisomerase IIα. It is of interest that these mutations appear to cluster in two "hotspot" regions, one near the putative ATP binding domain, and the other near the active site tyrosine that forms a covalent linkage with the DNA during the DNA cleavage reaction [95]. Similar mutations have been introduced in the yeast gene and even in the human enzyme expressed in yeast, and many of these mutations appear to confer resistance to topoisomerase II inhibitors [96, 97]. Hence, at least some mutations result in functional drug resistant enzymes.

The considerations in the previous section suggest that resistance to inhibitors of topoisomerase II can also arise by decreased expression of the enzyme. In principle, alterations in *trans*-acting factors can reduce the expression of several copies of a topoisomerase gene with a single change. Since topoisomerase I acts as a monomer, dominant resistance by mutation of topoisomerase I should not be possible, hence alterations in expression may be especially important with this enzyme. The basis for decreased expression of topoisomerase I or topoisomerase II is not known, but some studies have identified elements in the topoisomerase IIα promoter that alter the gene's transcription [98], and efforts are underway in several laboratories to identify *trans*-acting factors involved in this regulation.

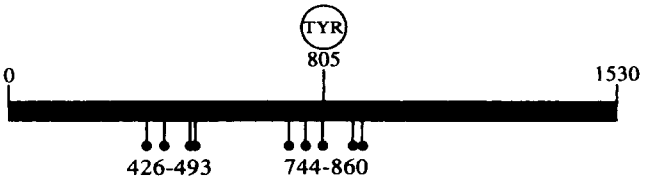

Figure 3. Location of topoisomerase II α mutations in the topoisomerase IIα structural gene. A listing of the precise mutational changes can be found in Beck [94]. As described in the text, the mutations identified thus far cluster to two regions (hotspot regions) of the topoisomerase protein. The location of the tyrosine that forms the covalent complex with DNA is also indicated.

What are the consequences of decreased topoisomerase activity in drug resistant cells?

How do topoisomerase mutations affect sensitivity to other drugs? In yeast, *top1* null mutations, which are insensitive to camptothecins, are hypersensitive to both complex stabilising and catalytic inhibitors of topoisomerases [44]. The converse also holds: reducing topoisomerase II to approximately 10% of wild type levels gives full resistance to complex stabilising topoisomerase II drugs and hypersensitivity to catalytic inhibitors of the enzyme [59]. Hypersensitivity to camptothecin is also observed when topoisomerase II activity is reduced [59]. In some cases, the reduced topoisomerase I activity leading to camptothecin resistance results in higher levels of topoisomerase II. Conversely, resistance to antitopoisomerase II agents is sometimes associated with high topoisomerase I levels. Cell lines selected for resistance to the complex stabilising drugs, such as teniposide and amsacrine, remain sensitive to such catalytic inhibitors of topoisomerase II, such as merbarone [40].

at-MDR and perturbations of pathways involving cytotoxic signalling, cell cycle, and programmed cell death

Mammalian cells with reduced levels of topoisomerases (either topoisomerase I or topoisomerase II) have no obvious phenotype, except that they tend to grow somewhat more slowly than wild type cells, as is common for many different drug-resistant cells [99]. However, just as topoisomerase-mediated cytotoxicity is, as discussed, an intricate and complex process, resistance to antitopoisomerase agents is likely to depend on changes in several different steps of the pathway. For example, since cell killing by camptothecins requires DNA synthesis, alterations in proliferation rate or cell cycle distribution could generate resistance to camptothecins. Alterations in pathways related to cell death represent another mode of resistance, since cells from transgenic mice lacking p53 are relatively resistant to antitopoisomerase II agents [73].

Work from several laboratories has shown that the proto-oncogene, *c-jun*, is induced in response to treatment of tumour cells with DNA damaging agents, including topoisomerase inhibitors [100–102]. Similarly, Kim and Beck found that this response was attenuated in cell lines that had been selected for resistance to teniposide [102]. Their results suggest that drug resistant cells may fail to activate signalling pathways that are activated in drug sensitive cells, or that drug resistant cells may activate other signalling pathways that are not activated in drug sensitive cells. One could speculate that drug resistant cells are partly defective in activating pathways that lead to cell death. It will be important to delineate the apparent differences in signalling pathways between drug sensitive and resistant cells.

IS THERE CLINICAL EVIDENCE FOR RESISTANCE ASSOCIATED WITH EXPRESSION OF ALTERED TOPOISOMERASES?

While the various possibilities for resistance to topoisomerase inhibitors have been considered, it is worth noting that to date, little or no evidence of altered topoisomerases has been found in clinical specimens. For example, in some studies [103–106], various leukaemias and solid tumours were analysed for decreased expression of topoisomerase II protein either by immunoblotting or by immunocytochemical staining, but no conclusions could be drawn. However, increased topoisomerase I activity has been shown to be associated with colon carcinoma compared to normal colon tissue, and elevations of topoisomerase II in some cancerous tissues have also been described [107]. In other studies [106 108], leukaemias were analysed for mutation by either SSCP or SSCP and direct sequencing of the "hot spot" regions of topoisomerase IIα, but again no evidence for altered topoisomerase II was found, except in one case of an ALL/AML (acute lymphocytic/myelocytic leukaemia) lineage switch [109]. While these results suggest that mutation in topoisomerase IIα may not be a common finding in leukaemic blasts from drug-treated leukaemia patients, they must be interpreted with some caution. The analyses by SSCP only examined the two hotspot regions where mutations had been identified in a number of cell lines. Recent studies in yeast indicate that many different regions of the topoisomerase II gene can give rise to resistance [110, 111]. As described above, mutations inactivating topoisomerase II can also lead to drug resistance, and inactivating mutations should occur throughout the gene. Moreover, preliminary findings revealed two mutations (one in the hotspot region and a mutation in a different region) in a relapse specimen of small cell lung cancer (SCLC), out of 13 patients examined [112]. While this is a potentially important observation suggesting that mutations in topoisomerase II in SCLC may be more common than those in AML, in neither study was the baseline frequency of mutation known, nor is it known if the mutations are silent polymorphisms. Nevertheless, allowing for the caveats that (a) the entire topoisomerase II gene has not been sequenced in any but a few cases of AML [109], and (b) the mutation frequency is unknown, the infrequency of mutations in hotspot regions of topoisomerase II in clinical specimens suggests either that we are not looking in the right place or that clinical resistance to topoisomerase inhibitors is due to other alterations.

CONCLUSION

We have attempted to summarise here current knowlege concerning antitopoisomerase drug action and resistance. While a good understanding about the mechanisms by which the enzymes work is emerging, and much is known about the way certain classes of drugs inhibit enzyme activity, we know less about mechanisms of resistance to these agents. Moreover, our understanding of the mechanism(s) by which inhibition of topoisomerase II (or topoisomerase I) is translated into a cytotoxic event is only beginning to come into focus. As we learn more about the complex machinery comprising programmed cell death pathways and the signals that initiate these responses during the next few years, we will doubtless have a clearer picture of mechanisms of cytotoxicity and of resistance to topoisomerase inhibitors, and this will enhance our knowledge of the role(s) of topoisomerases in clinical drug resistance.

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