Circumventing Antineoplastic Drug Resistance: When Tumor Cells Just Say "No" to Drugs

David J. Kroll[1]

School of Pharmacy, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver CO 80262

PROLOGUE

Antineoplastic drug resistance represents a specialized case of drug tolerance since cells most responsive to therapy are destroyed. Clinically, drug resistance may be observed at the onset of therapy or is acquired later by a tumor cell population over time due to a combination of spontaneous genetic mutations and selection by cytotoxic chemotherapy. Numerous mechanisms of drug resistance have been elucidated from *in vitro* studies but their relevance to clinical drug resistance has only recently begun to be appreciated. Newly described drug resistance mechanisms in tumor cells are discussed to demonstrate the potential value of a basic science approach to this clinical problem; difficulties associated with extrapolation from the laboratory to the bedside are also discussed. Strategies have been developed to target resistance mechanisms with the goal of improving chemotherapy success. As a lecture topic, examples are used that encourage students to realize that while resistance mechanisms may be responsible for therapeutic failures, these mechanisms may also represent future drug targets.

INTRODUCTION

Throughout pathophysiology, any attempt at pharmacotherapy is always subject to a homeostatic re-

sponse from the body. By invoking this response, the body's goal is to maintain what it perceives as a normal setpoint. In many cases, however, repeated drug administration leads to a prolonged adaptive response, or tolerance, to drug action. Tolerance can result from pharmacokinetic or pharmacodynamic adaptation, or a combination of the two. In the specific case of chemotherapeutic drugs (antibacterial, antiviral, antiparasitic, and antineoplastic drugs), drug tolerance or resistance is complicated by the fact that the most responsive invading tumor cells or pathogens are destroyed by drug therapy. This negative drug selection inherently carries with it a high probability that tolerance develops, since the least sensitive cells survive cytotoxic therapy. With regard to cancer chemotherapy this is also coupled with the high spontaneous mutation rate of transformed cells, making antineoplastic drug resistance especially problematic.

Antineoplastic drug resistance actually encompasses two types of drug tolerance. *Acquired* drug resistance is tolerance that results from cycles of cytotoxic antineoplastic therapy. Using lung cancer as an example, up to 90 percent of small cell lung carcinoma (SCLC) patients initially respond to chemotherapy. Unfortunately, patients tend to relapse with tumors that are highly drug-resistant as quickly as six to nine months later. Less appreciated, but equally important, is *intrinsic* drug resistance, or tolerance that exists at the time of diagnosis prior to drug therapy. An example of lung cancer with a high degree of intrinsic resistance to initial therapy would be non-small cell lung carcinoma (NSCLC).

Identifying the mechanisms in the laboratory that underlie resistance to various antineoplastic drug classes has been extremely fruitful. A primary goal of these studies is to identify mechanisms which may be exploited by the use of chemosensitizing agents that reverse clinical drug resistance and improve the success of chemotherapy. However, these attempts reveal important cautionary lessons in the extrapolation of basic science information to the clinic, since many of these resistance mechanisms also serve a protective role for normal tissue. This goal of this article is to review selected novel drug resistance mechanisms which have the potential to be targeted, to maintain or even improve selectivity of antitumor action.

MECHANISMS OF DRUG RESISTANCE

Any biochemical adaptive mechanism which reduces the selective toxicity, and therefore the therapeutic index, of a chemotherapeutic agent can contribute to drug resistance. This is illustrated by the fact that dose-escalation with most antineoplastic agents is usually limited by some severe host toxicity such as myelosuppression or cardiac damage. Therefore, any successful strategy to reverse tumor cell drug resistance must not simultaneously increase host cell toxicity as well. One must additionally bear in mind the toxicological profile of the drug or combination of drugs in question. Reduction in the severity of one dose-limiting adverse effect which enables an increase in drug dose may also result in a secondary adverse effect now becoming limiting. While there exist theoretically many points for pharmacologic intervention in tumor cell drug resistance, the greatest challenge has been identifying strategies which do not compromise the selective toxicity of a given agent for neoplastic cells. The following are selected mechanisms of drug resistance, some relatively novel, and their respective reversal

strategies for which this challenge may possibly be met through our understanding of the biology of these adaptive processes.

Pharmacodynamic Mechanisms

Qualitative alterations in the structure of a drug target molecule or quantitative changes in its concentration are among the most classically described drug resistance mechanisms. Resistance to the antifolate, methotrexate, represents such a mechanism, and this was one of the first cases where an effective strategy was employed clinically to circumvent resistance. Amplification of the gene for dihydrofolate reductase (DHFR), the target for methotrexate and other antifolates, leads to overproduction of this enzyme in resistant tumor cells(1). Increased levels of this target for these competitive DHFR inhibitors requires that more drug be administered. However, antiproliferative side effects such as myelosuppression and mucositis may then become dose-limiting. The same net effect also occurs when qualitative mutations occur in the structural gene for the enzyme that increases the K_i for the antifolates. However the well-established strategy of "leucovorin rescue," carefully timed administration of the preformed folate cofactor N^5-formyl-tetrahydrofolate after methotrexate infusion, protects host cells from methotrexate toxicity. This tactic allows methotrexate doses to be escalated by nearly two orders of magnitude. Hence, intracellular drug levels can be attained which inhibit DHFR activity despite the increases in enzyme level or K_i in the resistant cells. The key to the now widespread utility of this approach is that methotrexate administration can be increased without significantly compromising the therapeutic index of this drug, since host cells are in effect "rescued" by the leucovorin.

Another type of resistance-conferring alteration in an enzyme drug target can occur with the DNA topoisomerases. Topoisomerases are a group of enzymes which regulate the three-dimensional structure of DNA and play important and often essential roles in DNA replication, transcription, and mitosis(2,3). Topoisomerases are subdivided into two major groups. Type I topoisomerase is targeted by camptothecin analogues such as topotecan, which is currently under clinical evaluation. More clinically relevant are the type II topoisomerases which are targeted by etoposide, its analogue teniposide, and the aminoacridine derivative amsacrine. Anthracyclines such as doxorubicin and anthracenediones such as mitoxantrone both have as part of their cytotoxic action the ability to target topoisomerase II.

The mechanism by which resistance develops to anti-topoisomerase drugs is distinctly different from the antifolates. Nearly all anti-topoisomerase drugs used clinically are not competitive enzyme inhibitors, but are rather reversible poisons of the enzyme(2,3). Both topoisomerases modulate DNA topology via a catalytic cycle which includes a DNA cleavage event and formation of a transient intermediate in which the enzyme becomes covalently linked to the DNA through a phosphotyrosine bond(2,3). After passage of a single (type I) or double (type II) strand of DNA through the cleaved DNA, each enzyme reseals the break and dissociates from the DNA. This resealing or relegation event is the step which is inhibited by the anti-topoisomerase drugs, which covalently traps the enzyme on the DNA in its normally short-lived intermediate form. This stabilized ternary complex of drug, DNA, and enzyme may act directly as a barrier to DNA replication or indirectly as a trigger for programmed cell death (see below). Hence the anti-

topoisomerase drugs convert these important enzymes into lethal instruments and in fact require the presence of their respective topoisomerase target for their cytocidal antitumor effect to be expressed.

In contrast to resistance to competitive inhibitors of DHFR, anti-topoisomerase drug resistance can result from decreased levels of the enzyme in tumor cells(4-6). In support of this mechanism, resistance to one anti-topoisomerase agent often confers cross-resistance to other drugs which target the same topoisomerase enzyme. This phenomenon is termed atypical multidrug resistance [at-MDR] (7) since it differs from classical MDR which involves enhanced cellular efflux of drug (discussed below). Increased levels of a topoisomerase would increase the antitumor effect of drugs directed against that particular enzyme since more covalent drug-DNA-enzyme lesions could be produced(8,9). In fact, the selective antitumor effects of topoisomerase II poisons is partly due to the fact that rapidly proliferating cells contain high topoisomerase II levels while normally differentiated tissue contains very little topoisomerase 11(5,10).

Whether or not clinical resistance to anti-topoisomerase drugs develops as a result of reduced tumor cell content of each topoisomerase is only beginning to be studied(11). Strategies to selectively stimulate topoisomerase synthesis in tumor cells without accelerating proliferative rate may be a potential avenue for circumventing this drug resistance. For example, both granulocyte-colony stimulating factor (G-CSF) and hyperthermic treatments independently increase tumor cell topoisomerase II content in culture and, in turn, increase the tumoricidal effects of drugs like etoposide(12). Our own recent work has demonstrated that some experimental compounds used in leukemia differentiation therapy have the potential to stimulate topoisomerase II production during a very distinct time window(13). Differentiation therapy, the use of compounds like retinoic acid to block proliferation and induce terminal maturation of leukemia cells, has clinical utility when combined with conventional cytotoxic anticancer therapy (14). Therefore, carefully timed addition of a topoisomerase II-directed drug to such a regimen may effectively combat anti-topoisomerase II drug resistance in some leukemias. These laboratory approaches are however in their infancy and must be carefully evaluated in preclinical models to insure that host toxicity is not concomitantly increased. In addition it should be appreciated that other mechanisms beyond the scope of this discussion, such as structural mutations in the topoisomerase II molecule, can also contribute to the at-MDR phenotype.

Certain characteristics of *in vivo* tumor growth may also contribute to antitumor drug resistance. A very provocative series of experiments suggests that alkylating drug-resistance may be a function of cell-cell interactions in the three-dimensional structure of a solid tumor(15-17). For example EMT-6 mouse mammary carcinoma cells can be selected for alkylating drug resistance when maintained in mice, but when the same drug-resistant cells are grown in monolayer cultures the resistant phenotype is no longer evident(15). In culture, growth of cells as three-dimensional spheroids also appears to confer this resistant phenotype after only a single exposure to drug, and resistance cannot be accounted for by impaired drug penetration of the multicellular aggregate (16). Precisely how reconstitution of tumor architecture allows drug resistance to be expressed is presently unclear. How-

ever, it is well-known that reconstitution of basement membrane components in culture or *in vivo* propagation of cells has dramatic effects on cellular gene expression and morphology(17). Therefore, elucidation of the mechanism(s) underlying "multicellular drug resistance" will certainly be crucial to our understanding another pharmacodynamic modulator of antitumor drug response.

Cellular Pharmacokinetics

As with many pharmacologic agents, antineoplastic drug efficacy and selective toxicity is subject to the constraints of systemic absorption, distribution, metabolism, and excretion. Since most antineoplastic drugs exert their tumoricidal effects at intracellular targets on cellular molecules, drug disposition by the tumor cell itself becomes another consideration in drug resistance.

Probably the most widely studied resistance mechanism resulting from decreased intracellular drug accumulation is due to overexpression of P-glycoprotein or MDR1, the *mdr1* gene product(18). MDR1 protein is a member of a family ATP-dependent, transmembrane transporter proteins that facilitates the efflux of hydrophobic, heterocyclic antineoplastic drugs such as doxorubicin, vincristine, taxol, and etoposide. Tumor cell lines can be selected in culture with one of these agents, producing a cell line which expresses high levels of MDR1 protein. These cells then display pleiotropic cross-resistance to many other drugs which can also be exported by MDR1(19). Introduction of the MDR1 gene to tumor cells which normally lack this protein also confers resistance to these vastly different drugs(20).

Whether MDR1 overexpression is a clinically significant resistance (either intrinsic or acquired) mechanism is currently the subject of controversy(21, 22). Nonetheless, several phase I and phase II trials are underway to evaluate modulators of the MDR1 transporter (such as cyclosporin A, verapamil, quinine, and GG918) in an attempt to increase intracellular antitumor drug concentrations in resistant cells by inhibiting drug efflux. While this approach has been largely unsuccessful for solid tumors, response rates in phase II trials have been more promising for hematologic malignancies such as multiple myeloma, acute leukemia, Hodgkin's disease, and non-Hodgkin's lymphoma(23-25).

However, phase I trials with MDR modulators and chemotherapeutic agents have raised the quest) on of whether the increased response rate is due only to attenuation of tumor MDR1 protein function. For example, administration of cyclosporin A roughly doubles the AUC for etoposide(26) in addition to blocking etoposide efflux from MDR1-expressing resistant tumor cells. The inhibition in etoposide plasma clearance is likely due to the fact that the liver and kidney both normally express MDR1 protein; in fact, hyperbilirubinemia and renal insufficiency result from the fact that this normal host cytoprotective mechanism is also compromised by the MDR modulator. This observation has led to the suggestion that etoposide dosing be reduced by 50 percent in such regimens(26). In future phase II studies, however, response due to MDR modulator must ultimately be evaluated using equitoxic doses of antineoplastic agent to adequately test these regimens(21).

A major criticism of an ubiquitous role for MDR1 in clinical drug resistance is that MDR1 overexpression and resistance is infrequently observed in human malignancies. In fact, cells which express very low levels of MDR1 may

often be quite resistant to cytotoxic drug therapy. Therefore, alternative resistance mechanisms have been suggested. For example, another transmembrane, ATP-dependent transport protein termed MRP (for multidrug-resistance protein) has recently been cloned from a multidrug-resistant human SCLC cell line(27). Surprisingly, MRP is a rather distant relative of MDR1 and is instead more similar to the cystic fibrosis transmembrane conductance regulator, CFTR. Furthermore, MDR1 modulators such as cyclosporin A are unable to reverse the resistance of MRP-overexpressing cells lines. Data is equivocal as to whether MRP acts as a drug efflux pump like MDR1; explanations for MRP's mechanism of conferring resistance have focused on whether the protein simply sequesters drugs to prevent them from reaching their intracellular targets. Interest in MRP as a causative factor in human drug resistance has come from a very recent study of acute myeloid leukemia patients (AML) with an inversion in chromosome 16(28). Inversion 16 normally carries a relatively favorable prognosis. The MRP gene is located near the breakpoint of this chromosomal inversion and, in a subset of AML patients, the MRP gene has actually been deleted. Inversion 16 AML patients with MRP deletion from at least one chromosome were shown to have a significantly more favorable prognosis than their counterparts having both copies of the MRP gene(28). Loss of the MRP gene somehow enabled subsequent drug therapy to be more successful. This provocative study suggests that further elucidation of the role and mechanism of action of MRP in human multidrug resistance may reveal yet another target for resistance reversal/chemotherapy combinations.

While MDR1 and MRP-mediated drug resistance are likely caused by attenuated drug distribution to intracellular targets, accelerated cellular metabolism of the chemotherapeutic agents represents another resistance mechanism which may be taken advantage of clinically. Cytosine arabinoside (ara-C or cytarabine) is a highly effective in acute leukemias but has an extremely short half-life, and resistance can occur by an increased activity of the degradative enzyme cytidine deaminase(29). (Ara-C resistance is more commonly due however to reduction in cytidine kinase activity, which is required to activate ara-C (29)). Inhibition of ara-C degradation with deaminase inhibitor, tetrahydrouridine, has been proposed but this approach may also increase host toxicity, particularly since the liver is a major site of cytidine deaminase. The relatednucleoside analog, fludarabine, combines such an approach into the drug molecule itself; its fluorine substitution on the base renders the drug less susceptible to metabolism by adenosine deaminase.

Altered metabolism in drug-resistant tumor cells may be taken advantage of if an enzyme activates some drugs, while detoxifying others. An example is the quinone reductase DT-diaphorase (DTD) which activates mitomycin C to its DNA cross-linking species via a 2-electron reduction (30). Cells containing relatively high levels of DTD activity are most sensitive to the cytotoxic effects of mitomycin C(31,32). Conversely, decreased DTD activity can cause mitomycin C resistance. In a cell line selected for mitomycin C resistance which has less than 1 percent the DTD activity of its parental counterpart, very significant hypersensitivity to doxorubicin was observed(33). After ruling out a variety of other explanations, the investigators suggested that this hypersensitivity was a result of reduced DTD activity. Since doxorubicin can normally be metabolized to a less cytotoxic deoxyaglycone by DTD, reduced activity of DTD in a detoxification role likely increased the intracellular half-life of doxorubicin in this mitomycin C-resistant cell line. Although tumor cell populations are rarely as homogeneous in tumors as they are in culture, this example illustrates that careful understanding and comparison of antineoplastic drug metabolism and the major metabolic phenotype of a tumor may provide the basis for more rational chemotherapeutic drug selection.

DNA Repair

Another mechanism by which tumor cells can evade certain DNA damaging chemotherapeutic agents is by enhancement of their DNA repair capabilities. Covalent modification of DNA at the O^6 position of guanine is the primary cytotoxic lesion inflicted by many alkylating agents that have among the greatest (although still relatively poor) efficacy toward malignancies of the central nervous system. The DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGAT), can repair this lesion, rescuing the cell from cytotoxicity. Tumor cells can be classified into two groups depending on their relative level of AGAT. Cells with little or no AGAT activity are quite sensitive to alkylating agents. Cells containing relative high levels of AGAT activity are consequently resistant to these drugs. The latter phenotype may be partly responsible for both intrinsic and acquired resistance to the alkylating agents. Based on this tenet, a strategy was devised wherein inhibitors of AGAT were administered concomitantly with alkylating agents with the goal of overcoming resistance by changing the tumor cell DNA repair phenotype(34,35).

The most effective group of AGAT inhibitors are the O^6-alkyl substituted guanines, of which O^6-benzylguanine has been the most promising(35). These agents rapidly act as suicide substrate inhibitors of the enzyme, requiring new enzyme synthesis by the cell to reverse their effect. In vitro, O^6-benzylguanine is the most effective potentiator of the cytotoxicity of nitrosoureas such as BCNU, CCNU, but only in cell lines containing high levels of AGAT(35). The cytotoxic effect of alkylating agents which do not produce an O^6-guanine lesion (cisplatin and 4-hydroperoxylophosphamide) is not potentiated by O^6-benzylguanine. The crucial question is whether AGAT inhibitor therapy would also increase host toxicity of the nitrosoureas. When sensitive and resistant CNS tumor xenografts were grown in athymic mice and treated with B CNU, however, O^6-benzylguanine completely restored BCNU tumoricidal activity without increasing morbidity over a moderate dosing window(34). More comprehensive toxicological evaluation of this regimen prior to initiation of phase I trials is obviously indicated. A potential concern arises from a report that mutations can occur in the AGAT gene which reduce the efficacy of O^6-benzylguanine without compromising the DNA repair activity of the enzyme(36). This emphasizes the fact that cancer cells can become resistant even to resistance reversing agents. Nonetheless, such a strategy may still have utility in overcoming intrinsic and acquired resistance to some alkylating agents in the clinical setting.

Other cellular DNA repair processes may compromise the cytotoxic effect of DNA-damaging chemotherapeutic agents, but none have been studied to the point of AGAT modulation with regard to pharmacotherapeutic intervention. Future strategies may focus on novel DNA repair mechanisms only being elucidated recently. A particular exciting area has been the study of growth-arrest and DNA

damage *(GADD)* genes. The most widely studied *GADD* gene product, *GADD45,* is normally induced after DNA damage from either drugs or ionizing radiation and delays cell cycle progression at the border of G1 and S phase. This delay allows DNA repair to occur prior to DNA replication, thereby increasing the fidelity of DNA synthesis. Cells lacking this *GAD.D45*-dependent G1 checkpoint, such as in the cancer prone-disease ataxia-telangiectasia, are hypersensitive to DNA damage since they proceed to replication without adequate DNA repair(37). Regulation of the *GADD* response is complex, particularly with regard to its requirement for functional activity of the tumor suppressor gene p53 [(38) and discussed below]. Continued careful dissection of the biology of this cellular DNA damage response in particular is likely to reveal future drug targets which may be exploited in drug resistance reversal.

Altered Response

One cannot adequately discuss tumor cell drug resistance without mentioning the ubiquitous role of the tumor suppressor gene product p53. This protein was at first mistakenly thought to be oncogenic but closer evaluation revealed that p53 was actually mutated in a large proportion of human malignancies, silencing its tumor suppressor activity (39). Mutant p53 has been correlated with poor prognosis in many human cancers, but an absence of p53 mutations does not necessarily predict responsiveness to drug or radiotherapy since p53 function can be impaired by other mechanisms(37).

Numerous antitumor therapies (cytotoxic drugs and radiation) may ultimately kill cells via a single common pathway despite their wide range of intracellular targets. This pathway, termed apoptosis or programmed cell death, is a mechanism of so-called "physiological" cell death normally activated during development and tissue maintenance(40). Very diverse cytotoxic agents, at doses below those which cause general metabolic dysfunction, can trigger apoptotic cell death(41). In tumor cells, some types of apoptotic cell death require the presence of functional p53 protein. In what is likely to be a landmark paper in cancer chemotherapy, Lowe *et al.* (42) have reported that the cytotoxicity of agents as diverse as doxorubicin, etoposide. 5-fluorouracil, and ionizing radiation are all dependent on functional p53 in transformed cells. These *in vitro* findings have been subsequently confirmed in an *in vivo* animal model(43). This work carries several interesting consequences. When compared with non-transformed cells, oncogenic cellular transformation in conjunction with functional p53 appears to lower the threshold for a given drug's ability to initiate the cell death program. This observation probably contributes to the relative selectivity of antitumor action, and therefore the therapeutic index, of these such agents. Loss of functional p53 confers significant tumor cell resistance to these agents and may account for the poor prognosis of tumors exhibiting extensive mutation s in the p53 gene. In contrast, non-transformed cells are comparatively resistant to apoptotic induction regardless of p53 status. This latter point is emphasized by the fact that loss of p53 increases the chance that a cell may accumulate further mutations. As discussed earlier, increased mutational frequency may result since p53 delays the cell cycle at the G_1/S boundary after DNA damage through the *GADD* response(37). Mutations may increase the development of secondary drug resistance at the level of the drug target or the acquisition of a more aggressive growth phenotype.

Adding further complexity to this story is the fact that p53-mediated apoptosis can be overridden by expression of the bcl-2 gene(44), indicating an opposing drug response mechanism converging on the same pathway. In addition, apoptotic death which can occur via p53-independent mechanisms in other cell types can also be attenuated by bcl-2(45). It has therefore been suggested that "transcription-targeted therapies aimed at altering the expression of important apoptotic modulators such as bcl-2 or p53 downstream factors could provide powerful therapeutic options(46)."

Finally, the p53 tumor suppressor gene may have other activities which contribute to tumor progression when its function is compromised. Li-Fraumeni syndrome is characterized by a germ line p53 mutation and is classified by at least 3 first-degree relatives under the age of 45 having a sarcoma(47). Loss of p53 in fibroblasts cultured from Li-Fraumeni patients was accompanied by reduced expression of thrombospondin-1 (TSP-1), an endogenous inhibitor of angiogenesis (the production of new blood vessels)(48). Reintroduction of a functional p53 gene to these cells restored their ability to produce this angiogenic inhibitor, suggesting that p53 may also limit the expansion of supporting vasculature for tumor growth.

Angiogenesis

Nearly 25 years ago, it was suggested that production of a tumor blood supply by angiogenesis or neovascularization might represent another target for anticancer therapy(49). While one might initially think that increased perfusion of a tumor might permit more effective cytotoxic drug delivery, studies suggest that the high interstitial pressure of a tumor actually prevents drug diffusion from the circulation(50). In fact, a high density of microscopic vessels in a tumor, particularly in early-stage (I or II) breast carcinoma and pediatric brain tumors, is a negative prognostic indicator(51,52). Angiogenesis not only provides tumor nourishment but also provides a vehicle for tumor cell metastasis; for example, cellular expression of the endogenous angiogenic inhibitor. TSP-1, correlates inversely with metatstatic potential(53). Angiostatin, a recently discovered angiogenic inhibitor actually produced by primary tumors, can also suppress metastasis in a preclinical model(54). Taken together, angiogenesis can therefore be thought of as a mechanism of intrinsic drug resistance. Antiangiogenic therapy may be used as an adjunct to conventional chemotherapy after initial drug treatment, surgery, or radiotherapy(55).

Several polypeptide growth factors have been isolated that participate in normal and pathological angiogenesis by activating parent vessel endothelial cells to proliferate and invade surrounding tissue. These peptides are known as "direct" angiogenic factors since they are mitogenic for endothelial cells; other "indirect" factors may stimulate angiogenesis *in vivo* but are not themselves mitogenic. Proteases which can degrade the extracellular matrix also support angiogenesis and metastasis(56). The best characterized of the direct-acting factors are the family of basic fibroblast growth factors (bFGFs) and vascular endothelial growth factor (VEGF). These factors can be induced by indirect factors, but only VEGF is inducible by hypoxia(57).

Numerous agents have been developed to target these and other angiogenic factors(58) but most have only been evaluated in preclinical animal models due to the concern that these strategies may also inhibit coagulation and/or wound healing. However, several polysulfated compounds have been evaluated clinically for their ability to sequester

basic angiogenic factors, rendering them inactive. Suramin and pentosan polysulfate (PPS) are prototypes of this class. PPS appears to be the most promising from phase I trials since it can block angiogenic growth factor activity at one-tenth the plasma concentration that would delay coagulation(59). At the level of extracellular matrix degradation, tetracyclines (such as minocycline), tetrahydrocortisol, and the cyclodextrin derivative ß-cyclodextrin tetradecasulfate all have antiangiogenic activity. In a mouse lung carcinoma model, a combination of these compounds added to conventional cytotoxic therapy not only delayed tumor progression but also reduced the number and size of lung metastases(56). The semi-synthetic fungal product, AGM-1470, administered alone also suppressed the growth of several tumors in a mouse model system(55) and is now progressing to clinical trials. Surprisingly, another drug with antiangiogenic activity is the antiemetic/antileprosy agent thalidomide, a teratogen responsible for limb malformations in newborns in Europe during the early 1960s. Thalidomide is an effective inhibitor of bFGF-stimulated angiogenesis(60). This may explain its teratogenic effects in addition to revealing its potential utility in antiangiogenic therapy(60). Hence, continued pursuit of antiangiogenic approaches are likely to yield clinical successes in the near future.

SUMMARY

While this review has barely scratched the surface of factors underlying antineoplastic drug resistance, it is clear that the clinician faces innumerable obstacles in the management of malignant disease. For the most part, initial or acquired drug resistance *in vivo* is likely due to the interplay between permutations of these factors. Yet as drug resistance becomes a seemingly more complicated phenomenon, one can find hope in recent work suggesting a common resistance pathway (loss of apoptotic induction) that may be targeted, particularly if gene therapy approaches continue to develop. Antiangiogenic therapy also represents a high impact approach with the potential for intervening in the progression and metastasis of a vast group of solid tumors. Communication between the basic and clinical sciences is absolutely essential to the successful development of resistance reversal therapies. The interdependence between basic biochemical mechanisms and what can be achieved realistically in a patient cannot be overlooked if these strategies are to have utility in cancer treatment.

An Aside: General Comments on Teaching Methodology

With teaching commitments that include several other topics in pharmacology, one realizes that the lecture material in general requires some illustration to make complex concepts clear. Since students tend to remember images longer than they remember words(61,62) I also try to take advantage of contemporary anecdotal examples in the popular press to demonstrate basic pharmacological concepts, including those relating to drug tolerance. A favorite general drug tolerance example employs the late British punk rock musician Sid Vicious to demonstrate opioid agonist tolerance and considerations after opioid withdrawal in the tolerant individual. As was customary in his social circles, Mr. Vicious was a chronic parenteral abuser of heroin. Over time he developed tolerance to heroin's euphoric effects due partly to JJL receptor down-regulation, necessitating dose-escalation to achieve the same "desired" effect. When the musician was implicated in the stabbing death of his girlfriend, he was imprisoned. During this time he was opioid-free and his μ receptors likely increased to normal, pre-agonist levels. But when freed on bond, Mr. Vicious attended a party where he reportedly injected himself with an amount of heroin comparable to what he used when he was opioid tolerant, obviously without considering that the heroin would now be acting on a larger number of receptors, particularly in the respiratory center of the medulla. He subsequently died of respiratory depression due to opioid overdose before he could be tried for the crime.

This example serves a variety of educational purposes beyond simply demonstrating opioid tolerance. Storytelling as a general educational tool leads to a more conversational presentation and students tend to listen more closely(61). Such an example also gives students a familiar contemporary situation to discuss their coursework and career with friends and family from non-health care professions, individuals who might otherwise be intimidated or confused by science topics. Lastly such an example humanizes the instructor; that a pharmacology professor would even know who Sid Vicious is allows students to glimpse into the instructor's breadth of interests beyond the classroom. This general educational strategy has been proven effective in personalizing the large lecture class(63).

In discussing antineoplastic drug resistance, I may bring culture plates to my class representing the results of a clonogenic. cytotoxicity assay of a wild-type and drug-resistant tumor cell line exposed to identical etoposide concentrations. While plates with the drug-resistant line may contain thousands of crystal violet-stained colonies, others with the wild-type parental line may have only ten or so. These images of drug-resistance are far more reinforcing than simply displaying a dose-response curve representing this raw data. I also encourage students to peruse the popular press for examples to use in their own practice and in social discussions with the lay public. Locally, The Denver Post Science Today Section has recently covered such topics as the mechanism of action of topoisomerase-directed antitumor drugs and the potential role of MDR1 in drug resistance. Simple story-like examples open the door for our communication with peers and the public. As all of us, particularly at state institutions, come under legislative scrutiny with regard to our educational and health care missions, being able to convey to the public the job we perform as pharmacy professionals may become as significant as the job itself.

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