Clinical Drug Resistance: The Role of Factors Other Than P-Glycoprotein

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Clinical drug resistance is a major obstacle to successful chemotherapy for cancer. When it occurs, resistance to a wide range of agents is noted. This clinical observation should not be confused with so-called "multidrug resistance," which is a laboratory-based phenomenon, whereby cross-resistance in experimental models to structurally unrelated compounds is seen and is due to increased expression of P-glycoprotein (PGP). In the majority of cases of clinical drug resistance in solid tumors it is likely that other factors will play a major role. These other factors can be defined as pharmacologic or cellular. *Pharmacologic factors* are those that prevent an adequate degree of tumor cell exposure and include considerations of dose and schedule of drug, and also of drug metabolism, which may relate to concomitant medication and to genetic variations. Clinical maneuvers to circumvent drug resistance by increasing dose are so far of unproven value. *Cellular factors* are those that apply at the tumor cell itself, and it is probable that multiple mechanisms exist. These include considerations of drug uptake, activation/inactivation, and changes in target enzymes and in DNA repair processes. After DNA damage has occurred, a key determinant of the sensitivity of the tumor cell is its ability to undergo apoptosis. It is conceivable that failure to engage this process is a key factor in resistance to a number of drug classes, although there is little clinical evidence to support this at present. However, the genetic controls for the process of apoptosis are now being unraveled, and if this notion proves correct, the possibility will exist for the design of more rational means of circumventing drug resistance to a wide range of agents. In the meantime, strategies that should be pursued further in order to overcome this key clinical problem include further exploration of alternating or sequential drug schedules and using new non-cross-resistant agents, such as taxoids.

The use of drugs for treating cancer is a relatively modern phenomenon. Chemotherapy was first used in the 1940s following observations made during World War II.[1] Clinicians quickly learned that cytotoxic drugs are nonspecific poisons, but by skillfully manipulating drug schedules, it was possible to capitalize on the remarkable observation that recovery from the toxic side effects occurs more quickly in normal tissues than in certain chemosensitive cancers. It has become clear, however, that the number of solid tumors that possess such exquisite chemosensitivity is very limited, and for the majority of common solid tumors, this sensitivity to drugs is eventually lost. Although temporary further benefits can be obtained by retreatment with cytotoxic drugs, resistance to a wide range of agents generally becomes evident and is ultimately fatal. This phenomenon remains a principal obstacle to successful treatment, and for clinicians it is particularly frustrating. Contrast, for example, the management of a young man with testicular cancer with that of a patient with small-cell lung cancer. The response to treatment of widely metastatic testicular cancer is generally dramatic; more importantly, this is followed by permanent eradication of tumor and cure. In the case of small-cell lung cancer, however, impressive tumor responses are usually not maintained long-term, and relapse after several months (or possibly years) is the norm rather than the exception.

Clinically, the observation is that when drug resistance occurs, it applies to a wide range of structurally unrelated drugs. It is important that this clinical observation is not confused with the experimental observation that has been given the unfortunate title of "multidrug resistance." This latter observation is a fascinating experimental phenomenon whereby drug resistance to a range of natural products appears to be mediated through increased expression of the membrane transport pump known as P-glycoprotein (PGP). Since its discovery in 1976,[2] extensive studies have taken place to ascertain the clinical relevance of this phenomenon. This has been critically reviewed elsewhere,[3] but current information indicates that increased PGP ex-

pression could be relevant in the development of drug resistance in hematologic cancers, while its role in solid tumors seems much more limited. Interestingly, increased expression of PGP correlates with a worse outcome for a number of cancers, including breast and colon cancer.[4,5] However, there is little information from sequential studies to link this observation with cytotoxic drug resistance. Further studies are clearly required in order to clarify the prognostic importance and biological implications of increased PGP expression.

The growth of cancers is characterized by genetic instability.[6] This implies that during the development of tumors, heterogeneous populations of cells will expand, expressing varying degrees of chemosensitivity according to a range of cellular factors. The impact of chemotherapy will be to exert powerful selection pressures on these populations, allowing the outgrowth of the most chemoresistant. A further important factor is that as tumors grow, penetration of cytotoxic drugs into tumor cells may diminish because of changes in vascularity and oxygenation. Taken together, these considerations indicate that several factors are likely to impact on the development of clinical drug resistance; further, it is highly probable that a number of these will coexist.

WHAT ARE THE FACTORS UNDERLYING CLINICAL DRUG RESISTANCE?

Factors that are likely to be involved in clinical drug resistance can be divided into pharmacologic and cellular factors.

Pharmacologic Factors

A key determinant of chemosensitivity is adequate drug exposure at the site of action, i.e., the tumor cell. Drug exposure is a function of both drug concentration and time. The major factor that controls drug exposure is the treatment regime used, i.e., the dose and/or infusion duration, and this is generally limited by considerations of normal tissue toxicity. Differences exist according to drug type; for example, the peak concentration is more critical than duration of exposure for alkylating agents (e.g., cyclophosphamide), whereas the reverse is the case for phase-specific drugs, such as antimetabolites (e.g., methotrexate). Data from experimental models of drug resistance indicate the presence of a relatively steep dose–response curve (particularly for alkylating agents), and these have stimulated the design of various circumvention strategies. However, extrapolations to the problems of clinical drug resistance are not straightforward, although a number of clinical studies are now underway (see below).

TABLE I

Some Cellular Mechanisms Described in Experimental Models of Drug Resistance with Selected Examples

Mechanism	Drug	Reference
Defective/altered drug transport (including reduced receptor binding or membrane protein)	Methotrexate	[30]
	Alkylating agents	[19]
	Cisplatin	[19]
	Natural products	[31]
Reduced intracellular activation, or increased inactivation (e.g., by glutathione)	Cytosine arabinoside	[32]
	Cisplatin	[19]
	Anthracyclines	[33]
Reduced affinity for, or activity of intracellular target enzymes	Methotrexate	[34]
	5-fluorouracil	[35]
	Topisomerase I and II inhibitors	[36]
Increased repair capacity	Alkylating agents	[19]
	Cisplatin	[23]
	Nitrosoureas	[37]

Drug exposure may also be limited because of morphologic considerations, e.g., a tumor within the central nervous system, where the blood–brain barrier may prevent adequate exposure to pharmacologic intervention. However, a more general limitation applies to cancer cells within poorly vascularized regions of large tumor masses; suboptimal exposure results from limited penetration and is further complicated by the development of hypoxic areas that can reduce the efficacy of a number of cytotoxic agents.[7]

In many cases, adequate drug exposure at the tumor cell depends on conversion of the drug to its active form following administration. Examples include alkylating agents, which depend on hepatic metabolism, involving the cytochrome P450 enzyme system, to the active species. The level of activity of this enzyme system is subject to considerable interpatient variation, which may well be based on genetic differences.[8]

Cellular Factors

Assuming that optimal tumor cell exposure is obtained, a number of factors pertaining to the tumor cell itself may then be considered. These include (a) defective drug transport across the cell membrane; (b) enhanced drug inactivation or reduced drug activation; (c) altered levels of (or altered affinity for) a target enzyme; (d) enhanced level of repair of DNA damage. Examples for each of these have been identified for the major drug types using *experimental models* and are summarized in **Table I**; when drug resistance arises clinically, it seems likely that a number of mechanisms will come into play, but the extent to which these coexist has not yet been clarified.

For the reasons given previously, it is quite conceivable that no single cellular factor will be identified as being primarily responsible for any specific example of clinical drug resistance. However, it is now clear that the mechanism by which many structurally unrelated anti-cancer drugs have their effects involves the process of programmed cell death or apoptosis.[9] The failure of cancer cells to engage this process could underly resistance to a number of drugs. Various genetic factors have been identified that control the process of apoptosis, including a range of genes, such as p53, BCL2, and BAX.[10] The extent to which changes in levels of expression of any of these genes might provide a common mechanism for the development of clinical drug resistance requires urgent study.

As described above, a number of cellular factors have been identified as being relevant to the development of resistance in experimental models. These include cell lines derived from patients with drug-resistant tumors, as well as cell lines in which drug resistance has been derived experimentally by continued drug incubation in vitro. The resistance factors (the ratio between sensitive and resistant inhibitory drug concentrations) vary widely in these models, and for this reason in vivo models have been developed using both spontaneous murine tumors and human tumor xenografts. These can be useful for assessing means for modulation, but again their clinical relevance is unclear. A proper assessment of the cellular factors that underlie clinical drug resistance will therefore depend on an adequate body of information derived from clinical material. Ideally, tissue should be obtained from a cohort of patients before treatment and when disease relapses. Patients should all receive similar chemotherapy protocols, and full clinical follow-up needs to be available. There are few studies in the literature that so far fulfill all these criteria.

HOW CAN CLINICAL DRUG RESISTANCE BE CIRCUMVENTED?

Pharmacologic Resistance

A number of options exist for altering the route of administration of cytotoxic drugs, and these have the potential to circumvent drug resistance that is determined by limited drug access. Regional treatments, such as intrahepatic or intraperitoneal therapy, have been used extensively, and recent data do indicate an advantage for this approach in certain circumstances.[11,12] However, these will not address the issue of widespread systemic disease. An alternative approach is the use of high doses of chemotherapy, and modern techniques allow this to be employed safely because of the modulation of normal tissue toxicity. Examples include the use of peripheral blood stem cells to protect bone marrow

against the effects of myelosuppressive agents. Clinical trials are now addressing the issue of the importance of dose escalation, which can be achieved in this way, and results are eagerly awaited. This applies particularly to those agents, such as alkylating agents, where preclinical and clinical data indicate a positive correlation between increasing response and dose[13] (rather than duration). It seems likely that the benefits, if any, of this approach will be seen in its integration into the management of chemosensitive cancers, e.g., lymphoma,[14] rather than relatively resistant cancers, e.g., melanoma.[15]

Data already available do point to the potential limitations of dose escalation over a modest dose range. Randomized trials in ovarian cancer in which the dose of cisplatin has been doubled in one arm have shown that although response and median survival can be improved, long-term survival is not affected.[16] In order to make substantial improvements in treatment outcome, it may therefore be necessary to make much larger dose increments; using techniques mentioned above, doses can be escalated by a factor of at least 4 (for such drugs as carboplatin, etoposide, and cyclophosphamide) compared to standard regimens.

Another factor alluded to previously, i.e., the development of hypoxic cells within poorly vascularized tumor masses, can be addressed, not by dose escalation, but by specific drug design. Several forms of bioreductive agents, which are only activated to cytotoxic species in areas of hypoxia, have now been developed, and early clinical trials of novel structures are encouraging.[17] Such agents might find their greatest clinical utility in combination with radiotherapy (or other forms of chemotherapy) that would be capable of dealing with adequately oxygenated tumor cells.

Cellular Factors

Despite the lack of information from clinical material, a number of strategies are being pursued with the hope that at least some of the experimental data describing cellular factors underlying resistance do have clinical significance. A few examples of these are summarized in **Table II** and described below.

TRANSPORT: The development of analogues of existing cytotoxic agents has in some cases been based on improved *transport* properties. One example is the antimetabolite 10-EDAM (10-ethyl-10-dazaminopterin), which is an analogue of methotrexate. It shows enhanced transport into malignant cells, as well as enhanced polyglutamation and therefore reduced drug efflux. Phase I trials revealed dose-limiting diarrhea, leukopenia, and thrombocytopenia,[18] and Phase II trials have

shown activity in non-small-cell lung cancer and head and neck cancer.

ACTIVATION/INACTIVATION: In experimental models, such as ovarian cancer cell lines, resistance to alkylating agents and cisplatin has been clearly related to intracellular levels of glutathione, as well as metallathionen.[19] Glutathione (GSH) binds and inactivates such agents as cisplatin, and depletion of intracellular glutathione with the specific inhibitor of glutathione-S-transferase, buthionine sulphoximine (BSO), has been shown to reverse experimental resistance to melphalan and to cisplatin in both in vitro and in vivo ovarian cancer models.[20] Clinical trials with BSO have been initiated in Fox Chase Cancer Center and have confirmed the feasibility of obtaining significant reductions of intracellular GSH levels, as measured in peripheral mononuclear cells.[21]

TARGET ENZYMES: For a number of agents, reduced affinity for the target enzyme is a significant factor that limits activity. One of the main intracellular targets for 5-fluorouracil (5FU) is the enzyme thymidylate synthase, which is bound by the 5FU nucleotide 5FdUMP. The tightness of this binding is greatly enhanced if intracellular concentrations of reduced folate are increased, and this can be achieved experimentally by the addition of leucovorin.[22] Clinical trials of this approach have confirmed that the activity of 5FU can be enhanced in this way, and at least some elements of 5FU resistance may therefore be addressed.

DNA REPAIR: Experimental data indicate that enhanced repair of intracellular DNA adducts is involved in resistance to cisplatin and alkylating agents. A number of intracellular enzymes are involved in DNA repair, and inhibition of their activity clearly is a complex procedure. One of the enzymes involved is DNA polymerase (α and γ) and the agent aphidocolin is a specific inhibitor of this enzyme. In experimental models aphidocolin is capable of reversing cisplatin and alkylating agent resistance,[23] and Phase I trials of aphidocolin have demonstrated the feasibility of achieving steady-state concentrations of drug that are equivalent to those that are active in vitro.[24]

Alternative Strategies

The clinical significance of drug resistance has been appreciated for several years. One approach to circumvention has been to construct mathematical models that might lead to the generation of hypotheses that could be tested clinically. The most widely used of these is the Goldie-Coldman model, which was first published in 1979.[25] This is based on the assumption that drug-resistant cancer cells arise as a consequence of spontaneous mutation. The model allows the prediction of the probability of cure as a

TABLE II

Some Examples of Clinical Studies Aimed at Circumvention of Specific Resistance Mechanisms

Mechanism	Drug	Examples
Altered transport	Methotrexate	Methotrexate analogues, (e.g., 10-EDAM)
Activation/inactivation	Cisplatin Alkylating agents	BSO (glutathione depletion)
Altered affinity for target enzymes	5-fluorouracil	Leucovorin (binding to TS)
Increased repair	Cisplatin	Aphidocolin

function of the mutation rate to resistant cells and the total number of tumor cells present. The probability of cure equals $e^{-\alpha(M-1)}$, where e is the base of natural logarithms, α is the mutation rate per cell generation, and M is the total number of cells present in the tumor. Put simply, this indicates that the larger the tumor in terms of numbers of cells or, alternatively, the higher the mutation rate, the lower is the probability of cure. Intuitively, this seems logical, i.e., that smaller tumors are more likely to be curable and that treatment needs to be initiated as soon as possible for this reason. The second inference from this model is that the best chance of cure lies with the use of combinations of agents that are non-cross-resistant. These can be used in a number of ways, and one that has been advocated most widely has been the use of alternating sequences of non-cross-resistant combinations. A number of clinical trials have been pursued along these lines, and unfortunately the majority have proved negative. For instance, alternating sequences of chemotherapy in small-cell lung cancer,[26] advanced breast cancer,[27] and Hodgkin's disease[28] have indicated no clear evidence of superiority over conventional sequences. Part of the explanation may lie in the relative lack of non-cross-resistance of the combinations involved.

Over the past few years one of the most promising developments in new drug discovery has been the identification of taxoids.[29] These are agents that act to stabilize the microtubule, and they have wide activity in experimental models. Clear activity has been seen in drug-resistant models for both the taxoids that are now clinically available: paclitaxel (taxol) and docetaxel (taxotere). Most importantly, both these agents have demonstrated clear activity in the clinic in patients who have drug-resistant cancers, such as ovarian and breast cancer. Response rates in breast cancer have ranged up to 50% for patients whose disease is progressing on therapy with anthracyclines, whereas in patients with ovarian cancer whose disease is progressing on cisplatin or carboplatin, response rates for both

agents have been in the range of 20%. It is conceivable that these agents do represent truly non-cross-resistant drugs; for this reason, the strategy of employing alternating non-cross-resistant combinations that include taxoids should now be revisited.

FUTURE PROSPECTS

It is evident that clinical drug resistance is likely to relate to a number of factors that probably coexist. Circumvention is therefore likely to be a complex process and might well need the simultaneous use of strategies to overcome pharmacologic, as well as cellular, factors. It is generally accepted that DNA is the main target for many clinically useful drugs. For the future it will be important to pursue the notion that resistance to a number of these relates to failure of treated cells to engage the process of programmed cell death, or apoptosis. The genetic factors that control entry into apoptosis include the presence of functional p53 protein, the activity of which can be reduced by altered expression of members of other gene families, such as BCL2 and BAX. In addition, mutations of the p53 gene are widely seen in resistant cancer cells, and it is therefore possible that the failure of these cells to undergo apoptosis relates, at least partly, to inactivation of the p53 gene. Attempts to reverse this process experimentally are underway. Clearly, it is important to confirm that the failure of cells to undergo apoptosis is relevant to the clinical problem of drug resistance, and this will require careful studies using new functional assays. If these prove to be positive, it may then be possible to advocate methods for resistance circumvention that can be widely used and that may at least indirectly address the clinical phenomenon of drug resistance to a number of structurally unrelated drugs.

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