

MECHANISMS OF RESISTANCE TO ANTINEOPLASTIC DRUGS

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"Most patients with cancer demonstrate resistance (insufficient clinical tumor response) to the first chemotherapeutic agent or agents they receive."¹

This rather pessimistic quotation of Robert K. Oldham¹ forms the basis of this paper which briefly reviews the various bases of the apparent clinical resistance of cancers to drug therapy. This resistance is both the bane and the opportunity of all who are involved in cancer therapy.

Two general aspects of the problem of cancer cell resistance to drug therapy will be reviewed: firstly, a general discussion of the causes of tumor resistance to drug therapy and secondly, some examples of methods currently being tested to overcome this resistance.

Tumor resistance to chemotherapy can be the result of pharmacokinetic, cytokinetic or biochemical resistance. Pharmacokinetic and cytokinetic resistance are not traditionally viewed as being permanent since one can theoretically use a variety of techniques, such as regional therapy or dose scheduling, to overcome them. Conversely, biochemical resistance has frequently been viewed as permanent, but this view may be too gloomy, since novel ways of reversing this resistance are appearing in the literature on experimental chemotherapy.

Pharmacokinetic Resistance

"Pharmacokinetic sanctuaries" constitute one of the major categories of causes of drug resistance in tumors, particularly of those that are seen clinically. As the tumor cells proliferate, their blood supply is often marginal. The vessels are poorly formed and are sinusoidal, rendering blood flow sluggish. Under these conditions many cells become quiescent, or function only marginally because of the lack of oxygen and other nutrients and the buildup of metabolic by-products. Often these cells die and form necrotic masses that further complicate therapy. Drug penetration into sites where blood flow is marginal is poorer than it is into normal rapidly growing tissues where many of the major drug-limiting toxicities of cancer therapy are produced. Thus, it seems that one is able to deliver adequate amounts of drug where it is not wanted and inadequate amounts where it is needed.

Pharmacokinetic resistance can also be related to altered rates of absorption, biotransformation (activation or inactivation) and elimination of the anticancer drugs. These factors can vary between patients and can also be changed by simultaneous therapy with other drugs.

Cytokinetic Resistance

Cytokinetic considerations also play a considerable role in the success or failure of cancer therapy. Skipper & Schabel² published a table, originally proposed by Holland, dividing cancers into three classes according to their

curability by chemotherapy. This classification demonstrates the relationship between cytokinetics and tumor susceptibility to cytotoxic drugs. Tumors for which cures are achieved in more than 50% of cases, the "curable" tumors, have growth rates that are relatively rapid and there is a relatively high growth fraction. On the contrary, those for which cures are uncommon, the "precurable" tumors, have growth rates that are "less rapid" and the growth fraction is small. The intermediate "subcurable" tumors have a "less rapid" growth and a relatively low growth fraction. Furthermore, the influence of tumor size is critical to the success of therapy for "subcurable" and "precurable" tumors, whereas success is moderately independent of size for the "curable" tumors.

The key factors appear to be the growth rate and the growth fraction which are relatively high in "curable" tumors. The majority of currently used cytotoxic drugs are most toxic for cells that are traversing the cell cycle. Some, for example the antimetabolites which interfere with DNA synthesis or the vinca alkaloids which interfere with mitosis, are cytotoxic only when present in the stage in which they act. Thus cells that are not actively traversing the cell cycle, i.e., are in the G₀-phase, or even those cycling cells that are not in the appropriate cell cycle stage when these drugs are given escape significant damage.

Cytokinetics is also an important consideration in scheduling doses of certain drugs. In contrast to classes of cytotoxic drugs such as the alkylating agents where the effects of multiple doses are cumulative and dose scheduling does not appear to be critical, scheduling can be crucial with the antimetabolites.³ Giving mice cytarabine once daily for four treatments at a combined total dose of 960 mg/kg was not effective, whereas a combined total dose of 480 mg/kg subdivided into 8 doses on each of the same four treatment days as the first group was completely efficacious in curing the L1210 leukemia cell tumors. Thus, when the antimetabolite was continuously available throughout a 24-hour treatment period, and hence caught many cells as they entered the S-phase, it was much more efficacious than it was when one massive dose was given once during the day. Only those cells actually in S-phase while the drug was present were affected, and this usually represents only a small fraction of the stem cell population present.

Tumor cell heterogeneity may also be due to factors other than cell cycle stage. It is now apparent that there is also considerable heterogeneity in the stem cells themselves.² Various categories of cells could be described as belonging to compartments based on their sensitivity to cytotoxic agents. Drug sensitive cells traversing the cell cycle could be referred to as belonging to compartment "A". For a variety of reasons, possibly related to oxygen supply and nutrient status, these cells may move to compartment "B" during which they are resting and temporarily resistant to cell-cycle-specific drugs. Under proper circumstances, these cells can be recruited back into compartment "A", e.g. after a course of cytotoxic drug therapy reduces the size of the tumor due to its action on the stem cell population, or after administration of an effective "biological modifier." Both of these compartments can also give rise to "differentiated" cells or "dead" cells (compartment "C") that contribute to tumor mass but are not, in themselves, a problem, nor are they drug sensitive.

Cells from compartment "A" can also permanently develop specific or multiple drug resistances to move to compartment "D". Drug-resistant cells can move between resting and cycling states, as do drug-sensitive cells, but do not, under normal circumstances, revert to drug-sensitive status. We shall discuss the biochemical mechanisms of so-called permanent drug resistance later.

Cells that are temporarily resting or resistant (Compartment "B") constitute a larger fraction of the tumor mass in slowly growing tumors than in rapidly growing ones. Skipper and Schabel² also believe the ratio of permanently resistant (TR) to sensitive or possibly sensitive cells (TO) is likely to be higher in slowly growing tumors than in rapidly growing tumors of the same size. If the TR:TO ratio becomes greater than 1 during treatment, due to selection, the tumor will appear to become resistant to the therapeutic regimen.

Biochemical Resistance

There are two basic mechanisms by which an originally responsive tumor becomes resistant to a therapeutic regimen. The first assumes that all tumors contain at least a small proportion of cells permanently resistant to a particular drug by the time it is diagnosed, since it probably already has on the order of a billion cells. Thus, what appears to be de novo development of resistance is nothing more than selective killing of sensitive cells so that only resistant ones remain. On the other hand, in every tumor there is a finite rate of mutation, that is unaffected by most drugs, although it may be enhanced by the alkylating agents and other drugs that disturb DNA structure. If the mutation rate is relatively rapid and there is a large stem cell population in the tumor, the probability that a resistant clone will be formed increases. Just as before, the therapy can select for this resistant clone so that it becomes dominant and the tumor becomes macroscopically resistant to the regimen.

The biochemical mechanisms of drug resistance in cancer cells are reminiscent of those of bacteria against antimicrobials. Curt, Clendeninn and Chabner⁴, from whom this selection on biochemical resistance was adapted, list the following mechanisms: defective transport; defective drug activation; altered intracellular nucleotide pools; increased drug inactivation; altered DNA repair; gene amplification; and altered target protein. This excellent review should be consulted for additional detail and insight.

Defective transport can only be a problem for drugs that enter or leave the cell by carrier-mediated transport systems. This is never a mechanism of resistance of the nitrosourea alkylating agents. Defective transport can be due to decreased carrier-mediated drug uptake (e.g. methotrexate and mechlorethamine); decreased membrane nucleoside transport-binding sites (e.g. cytarabine); and increased efflux of drug from the cells (e.g. doxorubicin).⁴

Methotrexate is the prototypical example of an anticancer drug that enters the cell by specific carrier-mediated uptake. When this transport mechanism is absent, insufficient methotrexate enters the cells to adversely affect the dihydrofolate reductase and the tumor is unaffected. It has been shown that when the extracellular concentration of methotrexate exceeds 10 micromolar, effective intracellular concentrations are achieved by passive diffusion.⁴ The

observation that massive doses can circumvent the absence of this transport system forms the basis of high-dose methotrexate treatment with leucovorin rescue. As an aside, the effectiveness of high-dose methotrexate with leucovorin rescue is being questioned.⁵

It is of interest that melphalan, sometimes called phenylalanine mustard, enters cells by the leucine transport system. Mechlorethamine, nitrogen mustard, enters cells by the choline transport system.⁴ Since these alkylating agents enter the tumor cells by different mechanisms, it is not surprising that resistance to one does not automatically imply that other alkylating agents will be ineffective.

Defective drug activation results in failure of many anticancer drugs which are actually pro-drugs. This seems to be especially characteristic of antimetabolites, many of which must be metabolized to phosphorylated and/or to deoxy-pentose derivatives to be active. Specific examples include decreased hypoxanthine-guanine phosphoribosyl transferase activity (e.g. 6-mercaptopurine and 6-thioguanine), decreased activity of multiple activating enzymes (e.g. 5-fluorouracil), and decreased cytochrome P-450 or flavin reductase (e.g. doxorubicin).⁴

One of the many hypotheses advanced to explain the mechanism of action of doxorubicin is that it is reduced to the semiquinone derivative. This derivative can form free radicals which can either directly damage DNA and proteins or it can lead to oxygen free radicals which also damage cellular constituents. Some resistant cell lines have been observed to have decreased cytochrome P-450 or flavin reductase activity which would lead to a decrease in the rate of semiquinone formation.⁴

Defective drug activation also hampers the efficacy of cytarabine, 5-azacitidine and methotrexate, all of which are antimetabolites. The methotrexate example is especially interesting since its recognition awaited the understanding that inhibition of the enzyme dihydrofolic acid reductase was not its sole mechanism of action. Methotrexate can be incorporated into gamma-polyglutamate derivatives normally present in cells, but the methotrexate-containing derivatives are cytotoxic. Failure to metabolize methotrexate to the polyglutamate derivatives has been observed in some methotrexate-resistant human tumors.⁴

Some tumors become resistant to cytotoxic drugs by increasing their ability to inactivate them. A membrane associated alkaline phosphatase with different characteristics than the one we normally refer to by this name is apparently induced in some resistant cells.⁴ The actions of 6-mercaptopurine and 6-thioguanine are decreased by increases in this enzyme. Increased catabolic enzyme activity in certain tissues may also explain decreased activity. For example, bleomycin hydrolase and cytidine deaminase activity have been shown to be increased in some tumors.⁴

A more general mechanism is an increase in the reductive capacities of the cell. This usually includes increased concentration of sulfhydryl-containing entities (such as glutathione or metallothionein) that scavenge active radicals in the cell. This mechanism may operate against some of our major cytotoxic drugs, such as the alkylating agents, cisplatin and doxorubicin and would be expected to confer some degree of cross resistance among these drugs.⁴

Increased efficiency of excision of nucleic acid bases damaged by covalent binding of alkylating agents, cisplatin or doxorubicin can account for some tumor cell resistance. Since these drugs can also cause breaks in the DNA chain, ligase activity is important as well.⁴ Advances in molecular biology are adding to our ability to appreciate and understand the importance of these steps as mechanisms of resistance.

Increased numbers of genes, either as part of a single chromosome or due to extrachromosomal DNA fragments, may lead to increased mRNA coding for certain enzymes.⁴ This may be responsible for increased concentrations of enzymes such as aspartate transcarbamylase, the target for (N-(phosphonacetyl)-L-aspartic acid and dihydrofolate reductase, the target enzyme for methotrexate. Such increases would increase the concentration of inhibitor required to effectively block the pathway just as it would if more enzyme were added to a test tube. In some instances, increased reducing activity may have a similar origin since increased amounts of metallothioneine have been observed in resistant cells.⁴

Altered targets have been reported to be causes of resistance to such drugs as methotrexate, vincristine, 5-fluorouracil, hydroxyurea, the steroids and doxorubicin. In some cases, the affinity of the receptor for the drug is decreased. In others, notably the receptors, the steps between drug-receptor combination and action may be defective.⁴

Drugs that ultimately interfere with nucleic acid synthesis may fail because of increased intracellular nucleotide pools (e.g. cytarabine) and increased efficiency of the salvage pathways (e.g. methotrexate and 5-fluorouracil). Improved access to precursors following the blocked step lessens the effect of blocking a specific enzyme. Thus, even the lower level of operation in the inhibited state produces sufficient de novo synthesis of the nucleoside required.⁴

Reminiscent of the transfer of resistance to several antibacterials in a single plasmid through conjugation, cancer cells can develop what is known as "pleiotropic drug resistance."⁴ Pleiotropic drug resistance refers to the development of resistance to drugs with different mechanisms of action and diverse chemical structures. Well-known examples include resistance to doxorubicin, dactinomycin and the vinca alkaloids. This resistance may be due to a single mechanism but at present this process is little understood.

Overcoming/Reversing Resistance

Serendipity and increased awareness of the various mechanisms of resistance are leading to more creative attempts to overcome or reverse it. For example, it has been shown that some agents that alter calcium movement in the cell can reverse pleiotropic drug resistance in some experimental tumors.⁴ Examples are calcium channel blockers (e.g. verapamil and diltiazem), calmodulin inhibitors (e.g. prenylamine and trifluoperazine⁶), and the old turkey tranquilizer, reserpine.⁴ Perhexiline maleate, reported to be a calcium antagonist, also reverses resistance to doxorubicin in a model system, but the investigators believed that the effect was due to alterations of cell lipid metabolism rather than calcium antagonism.⁷ No reports of clinical trials testing the efficacy of these agents are available.

Biological response modifiers constitute an important emerging area of investigation. Various biological modifiers, such as specific monoclonal antibodies to tumors, have increased tumor response to cytotoxic drug therapy.¹ If this is found to be generally true for a wide variety of tumors or for an important subset of a specific tumor type, it could represent a significant step forward. An excellent review of the variety and current status of biological response modifiers has been written by R.K. Oldham.¹

Participants in the Purdue Comparative Oncology Program (PCOP) (including Drs. Ralph Richardson, William Voorhees, Charles Babbs, William Blevins, Glenn Elliott, Steven Badylak, Jan Bartlett and William Carlton) are collaborating to study a variety of means of overcoming pharmacokinetic drug resistance. Studies on regional therapy include close intra-arterial and retrograde venous injection where drug distribution and blood flow are being monitored. Investigators elsewhere are studying intraperitoneal injections^{8,9}, which apparently provide superior efficacy for certain tumors. The PCOP is also studying the distribution and efficacy of doxorubicin encapsulated in ferritin-labeled albumin microspheres.¹⁰ These microspheres are injected into the tumor and held there by a powerful magnet. If held in place for approximately 20 minutes, the microspheres apparently do not leave the tumor and theoretically release their drug over a prolonged time. If successful, this would be a significant step forward because free adriamycin is not preferentially taken up by tumors as opposed to other tissues even when injected directly into the vasculature supplying the tumor.¹¹ Means of altering drug distribution and toxicity being attempted by other investigators include covalently binding drugs to microspheres and dextran¹² and encapsulation of various drugs in heat-sensitive liposomes¹³ followed by use of hyperthermia treatment of the tumor to release the drug.

Conclusions

Thorough understanding of the various mechanisms of tumor resistance to cytotoxic drugs can lead to rational approaches to overcoming it. Widening our horizons to be more aware of the normal processes by which the body fights cancer and the influence of the biological response modifiers on these processes should also be of tremendous benefit. Such knowledge would permit us to mix our armamentarium of cytotoxic drugs, biological response modifiers, surgery, radiation, heat, etc. optimally in the fight against cancer.

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