Chemosensitizers to Overcome and Prevent Multidrug Resistance?

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The emergence of multidrug resistance is a well-recognized and major obstacle to successful chemotherapy of cancer. While chemotherapy is often quite effective in treating certain cancers, including Hodgkin’s disease, large-cell lymphoma, acute lymphocytic leukemia, or testicular cancer, most other types of cancer respond to chemotherapy poorly or partially (i.e., an initial response to chemotherapy followed by a relapse associated with resistance to anticancer drugs). In the laboratory, multidrug resistance has been modeled by selecting cultured cells in the presence of a single cytotoxic agent (e.g., doxorubicin), which usually leads to cross-resistance to a variety of anticancer drugs that differ in structure and mechanism of action (e.g., anthracyclines, vinca alkaloids, taxanes, and epipodophyllotoxins). During the past two decades, two major directions of research on multidrug resistance have emerged: 1) the elucidation of the genetic and biochemical bases for the multidrug-resistant phenotype and 2) the development of effective new therapies to circumvent multidrug resistance.

Several differences have been defined between multidrug-resistant cells and their drug-sensitive counterparts. They may be broadly categorized as 1) reduced accumulation of cytotoxic drugs as a result of decreased drug influx and/or increased drug efflux, 2) altered expression and/or activity of certain cellular proteins, and 3) physiologic changes that alter the intracellular milieu. Depending on the anticancer drug used for selection, overexpression of a number of proteins has been associated with multidrug resistance in vitro: the multidrug resistance (MDR1; also known as P170) gene product P-glycoprotein (1,2), the multidrug resistance-associated protein Mrp (3,4), and the lung resistance protein LRP (recently identified as the major vault protein) (5,6) as well as several enzymes (7-9). In addition, multidrug resistance has been ascribed to reduced expression and altered activity of topoisomerase II (10-13) or altered expression and/or mutations in tubulin isoforms (14-16). Each of these proteins has been associated with a specific multidrug-resistant phenotype, and each may be viewed as a therapeutic target pending proof of clinical significance in drug-resistant human cancers.

Current strategies to circumvent multidrug resistance in the clinic have focused mainly on inhibiting expression and/or activity of the human MDR1 gene product, the best characterized mechanism for pleiotropic multidrug resistance. P-glycoprotein confers multidrug resistance by functioning as an efflux pump for a diverse assortment of anticancer drugs, including vinblastine, vincristine, doxorubicin, daunorubicin, paclitaxel (Taxol), etoposide, teniposide, and dactinomycin (2,17). There is now significant evidence supporting an association between P-glycoprotein expression in tumor specimens from patients and a poor

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treatment prognosis (18,19). Pharmacologic reversal of P-glycoprotein-mediated multidrug resistance in vitro was first reported by Tsuruo et al. (20,21), who demonstrated that verapamil or trifluoperazin enhanced the intracellular accumulation of vincristine, potentiating its antiproliferative activity in a multidrug-resistant murine leukemia cell line. Many chemosensitizers have since been reported that antagonize multidrug resistance in vitro, and some are effective in vivo upon coadministration with the appropriate chemotherapeutic agents to mice with multidrug-resistant tumors (22). Most of these multidrug resistance-reversing agents inhibit the drug efflux function of P-glycoprotein via direct interaction with the MDR1 gene product (e.g., the cyclosporin PSC 833), and some (e.g., verapamil and cyclosporine A) represent substrates for P-glycoprotein-mediated transport (2). Unfortunately, clinical studies with first-generation multidrug resistance modulators (e.g., verapamil and cyclosporine A) have indicated dose-limiting side effects of the chemosensitizers themselves, a failure to achieve therapeutic blood levels, or pharmacokinetic drug interactions that complicated patient dosing (23,24). Less toxic, second-generation multidrug resistance-reversing agents (e.g., dexverapamil and PSC 833) are currently being evaluated in clinical studies by several investigators (23,25). Recently, some encouraging data on patients with hematologic malignancies have been reported (25-27), supporting the potential of chemosensitizers in overcoming multidrug resistance in the clinic. On the other hand, results of studies in patients with solid tumors have been somewhat disappointing, leading some to question the validity of the multidrug resistance hypothesis, the reversing agents themselves, or the strategy for their most effective use (23,24).

The article by Beketic-Oreskovic et al. (28) appearing in this issue of the Journal takes the multidrug resistance question one step further by addressing the nature and mechanism of resistance of human sarcoma cells to the combination of an anticancer drug and a chemosensitizer. This important question has not received much attention to date, but it may be central to the optimal strategy for clinical use of multidrug resistance modulators. Beketic-Oreskovic et al. describe the results of a Luria-Delbrück fluctuation analysis of human MES-SA sarcoma cells, in which a single-step coselection was applied using doxorubicin (40 nM) and the multidrug resistance-reversing agent PSC 833 (2 μM), extending their findings from a previous study using selection with doxorubicin alone (29). With this type of analysis, it can be established that resistance is due to the clonal expansion of spontaneously mutated cells rather than to the induction of resistance by drug exposure (30-32). Beketic-Oreskovic et al. (28) present several key results in their article. First, coselection with doxorubicin and PSC 833 yielded drug-resistant variants with an approximately 10-fold lower mutation rate (2.5 × 10^{-7} per cell generation) than selection with doxorubicin alone (1.8 × 10^{-6} per cell generation). Second, while the drug-resistant variants selected with doxorubicin alone predominantly showed increased MDR1 gene expression, no MDR1-overexpressing clones were obtained from the coselection with doxorubicin and PSC 833. Third, the double-selected, drug-resistant variants exclusively exhibited alterations in expression and activity of topoisomerase IIα, a specific target protein of anthracyclines, shedding light on potential mechanisms of resistance that might develop during clinical use of a combination of doxorubicin and PSC 833.

These findings may be relevant to the design of clinical studies as discussed below; however, they also raise some important questions. How universal are these results? In particular, would a similar 10-fold difference in mutation frequency be observed after coselection with doxorubicin and a different chemosensitizer or with PSC 833 and another cytotoxic drug (e.g., etoposide, vincristine, or paclitaxel)? If the coselection was performed with a different class of cytotoxic drug, would the emerging mechanism of drug resistance consistently involve the intracellular drug target protein, as opposed to activation of a pleiotropic, membrane-associated mechanism (e.g., overexpression of MDR1 or MRP proteins)? In other words, would coselection with etoposide and PSC 833 also lead to altered topoisomerase IIα expression or activity? [A previous study by the Sikic laboratory using selection with etoposide alone (33) suggests that this is a likely result.] Moreover, would coselection with vincristine or paclitaxel and PSC 833 yield increased expression of certain tubulin isoenzymes and/or mutations in tubulin-binding sites? Would similar results be obtained in studies with other tumor cell lines that do not express P-glycoprotein, and what would the results be in coselection experiments with MDR1-positive cancer cell lines? Obviously, these questions are beyond the scope of one study, but they are relevant in any attempt to generalize from the data presented in the article by Beketic-Oreskovic et al. (28).

Another interesting aspect implied by the study by Beketic-Oreskovic et al. would be to experimentally address why the mutation rate is lower in the doxorubicin + PSC 833 double-selection system than in the selection system with doxorubicin alone. For both types of selection, the observed mutation rates are in the range for spontaneous mutations; however, it seems intriguing that all the random variants selected by doxorubicin + PSC 833 would involve topoisomerase IIα alterations, whereas random variants selected by doxorubicin alone predominantly showed activation of MDR1 gene expression. The simplest explanation (and one stated by Beketic-Oreskovic et al.) may be that the rate of mutations was in fact unchanged and that variants with activation of the MDR1 gene also occurred in the double-selection system, but that the relatively high, though clinically achievable, concentration of PSC 833 present in the selective media maintained sensitivity of these cells to doxorubicin cytotoxicity. This simple explanation is at the same time an attractive explanation that offers strong support for the use of an MDR1-reversing agent in preventing the emergence of the MDR1 phenotype in drug-sensitive tumor cells.

The observation that a drug target-specific resistance mechanism rather than a pleiotropic, membrane-associated mechanism emerges upon coselection with doxorubicin and PSC 833 may also have interesting implications. Topoisomerase II-related cross-resistance includes anthracyclines, the epipodophyllotoxins etoposide and teniposide, and ellipticine, but it leaves the cells sensitive to tubulin binders such as vinca alkaloids or taxanes. Although a caveat, emergence of a target-specific resistance mechanism may be more predictable and more manageable than emergence of a pleiotropic mechanism. Assuming that
this observation by Beketic-Oreskovic et al. will be substantiated in additional Luria-Delbrück fluctuation analyses performed with combinations of PSC 833 (or other multidrug resistance-reversing agents) and other classes of anticancer drugs, it may translate to the clinical use of combinations of cytotoxic drug and multidrug-resistant chemosensitizer. Cancers would remain responsive to certain classes of anticancer agents, and one could envision a “combinatorial” approach of chemosensitizer and cytotoxic drugs rather than the standard regimens to thwart “true multidrug resistance.”

Generally, clinical studies involving use of chemosensitizers are attempting to treat patients with established multidrug-resistant cancer (19,23-27). In most instances, patients enrolled in these studies have advanced disease, and several treatment regimens have failed. The elimination of an established or a metastatic multidrug-resistant tumor by chemotherapy proves difficult, and discouraging results from patients with solid tumors have generated considerable controversy about the effectiveness and broad potential of multidrug resistance-reversing agents. The in vitro findings of Beketic-Oreskovic et al., however, argue that use of chemosensitizers to prevent pleiotropic multidrug resistance may be a more appropriate strategy for assessing their clinical potential in cancer chemotherapy. When a chemosensitizer with an acceptable safety profile advances from current phase I/II studies, logic suggests this hypothesis should be tested clinically.

References


Note

Vertex Pharmaceuticals Incorporated, which employs both authors, has a multidrug resistance program. Neither the article by Beketic-Oreskovic et al. nor this editorial relates to the company or the program.