Drug Resistance: Still on the Learning Curve

Susan E. Bates

Medicine Branch, National Cancer Institute, Bethesda, Maryland 20814

For oncologists, the identification of the transporter MDR1/Pgp as a mechanism of drug resistance offered the possibility of finally succeeding in the treatment of patients with refractory tumors. Numerous laboratory studies in cell line models have shown that high levels of MDR1/Pgp can confer resistance levels of 1000-fold or more. These cell lines could be dramatically sensitized to chemotherapy by the addition of antagonists to inhibit Pgp-mediated drug efflux. These laboratory observations gave rise to very high expectations, and it perhaps should not be surprising that disappointment followed. Studies of the reversal of drug resistance with first-generation Pgp antagonists including verapamil, cyclosporine, and quinidine, among others, failed to convincingly show improved response to chemotherapy (1, 2). However, the fault was attributed to the lack of potency of the first-generation reversal agents, which were selected because they were already in clinical use for other indications. Second-generation antagonists, which were developed exclusively for their ability to overcome Pgp-mediated resistance, are still in development, although some of the early studies with PSC 833, VX 710, and GF120918 could also be added to the negative side of the balance sheet (3–5). After a decade of research, it seems clear that drug resistance, once established, is multifactorial and cannot be attributed solely to Pgp overexpression. It also seems clear that our experimental design must be altered. With some exceptions, we have attempted to treat tumors with Pgp antagonists only after the tumor has been repeatedly exposed to antineoplastic agents, a strategy comparable to a military commander staying the attack until the enemy side is heavily armed and fortified.

Instead, it can be argued that a strategy aimed at preventing the emergence of drug resistance is more likely to be successful. Several lines of evidence support this argument, including the data found in the report by Abolhoda et al. (6) in the current issue of Clinical Cancer Research that demonstrate that MDR1 expression can be acutely induced in human cancer by exposure to doxorubicin. Induction during treatment could provide the marginal decrease in intracellular accumulation that a cell needs to withstand the effects of chemotherapy.

The first line of evidence suggesting that preventing the emergence of resistance ought to be the goal of drug resistance reversal studies can be found in laboratory models that have demonstrated a striking advantage for the addition of a Pgp antagonist during the initial exposure of cancer cells to chemotherapy. Using a mutation rate analysis, Beketic-Oreskovic et al. (7) reported a 10-fold reduction in the emergence of resistant clones by exposing human MES-SA sarcoma cells to doxorubicin in the presence of the potent cyclosporin Pgp antagonist PSC 833 (Valspodar). Single-step treatment with 40 nm doxorubicin alone resulted in the selection of spontaneous drug-resistant mutants at a rate of 1.8 × 10⁻⁶ per cell generation. In the presence of PSC 833, mutations were detected at a rate of 2.5 × 10⁻⁷ per cell generation, and the resistant clones that emerged in PSC 833-treated cells had a reduced expression of topoisomerase IIα, rather than an overexpression of Pgp. These data clearly support the use of Pgp antagonists in the initial treatment of cancer to reduce the frequency of emergent drug-resistant clones.

A second line of evidence is derived from the results of a Southwest Oncology Group Trial in patients with poor-prognosis acute leukemia. In this trial, published in abstract form only, 226 patients treated with cytosine arabinoside and daunorubicin were randomized to concomitant treatment with the Pgp antagonist cyclosporin A or to no additional agent (8). Whereas no significant impact on complete response rate was noted (40% versus 33%), relapse-free survival at 3 years demonstrated a significant improvement (43% versus 10%; P = 0.033) for patients treated with the cyclosporin. In retrospect, it seems obvious that in chemotherapy-responsive malignancies, an emphasis on response rates is the wrong end point. For breast and ovarian cancer and for lymphoma and leukemia, response rates are already high and are difficult to improve. However, an unacceptable proportion of patients eventually experience disease recurrence. It can be argued that prevention of the emergence of resistance, as measured by prolonging the response duration and lowering the relapse rate, is the proper end point for studies of resistance modulators.

The report by Abolhoda et al. (6) in the current issue offers a third line of evidence. Patients underwent isolated lung perfusion with doxorubicin for treatment of metastatic sarcoma; tumor and normal tissue biopsies were performed before and 20 and 50 min after initiation of the perfusion. In four of five patients, tumor samples obtained after the perfusion demonstrated an increase in MDR1 expression. The increase ranged from 3-fold to 15-fold at the 50 min time point, with control lung tissue showing no change in gene expression. Although the data are limited (only five patients were studied), and the pre- and postinfusion samples represent different sites of metastasis, which could have different basal levels of expression, the results are intriguing. The studies suggest that acute induction of MDR1 expression can occur, a situation that could profoundly affect response to treatment. Furthermore, induction during treatment could have confounded previous studies that relied on the correlation of static, pretreatment Pgp levels to interpret the response rate.

Despite all that we know about MDR1 and Pgp, the most intensively examined mechanism of drug resistance, we know surprisingly little about how the gene is regulated. Differentiat-
ing agents, hormones, oncogenes, and transcription factors have been shown to modulate human and murine MDR1 expression. Agents or factors as diverse as sodium butyrate, retinoic acid, vincristine, raf, p53, nuclear factor κB, NF-IL6, and YB-1 have been implicated (9–16). For the majority of these studies, the evidence has been derived from transfection of reporter constructs containing various lengths of the MDR1 promoter; hence, one must be cautious in the interpretation of the data. However, we know that the endogenous gene can be modulated in animal models: exposure to carcinogens and partial hepatectomy induce MDR1 in murine liver (17); and steroid hormones induce MDR1 in the secretory epithelium of the uterus (18). Interestingly, nuclear levels of YB-1, a transcription factor up-regulated by conditions invoking cell proliferation, have been shown to correlate with MDR1 expression in human breast cancer and osteosarcoma (19, 20). Taken together, the data suggest that MDR1 activation can be triggered under a variety of conditions. This induction is reversible and would not be followed by up-regulation of MDR1 expression when the tumor tissue is biopsied subsequently. In such a case, MDR1 would contribute to resistance without detection in clinical assays.

Alternatively, the inducible mechanism may coexist with a fixed overexpression. One mechanism by which constitutive overexpression may occur is through gene rearrangement. Mickley et al. (21, 22) have shown that the MDR1 gene in drug-resistant cell lines has frequently been rearranged to come under the control of a constitutively active promoter. In two-thirds of cases, the new promoter is found elsewhere on chromosome 7, indicating an intrachromosomal exchange, whereas in the remaining cases, the new promoter is found outside chromosome 7. Evidence for MDR1 overexpression due to gene rearrangement has also been obtained in clinical leukemia samples. Because rearrangements more frequently involve actively transcribed genes, acute induction by chemotherapy, as suggested by Abolhoda et al. (6), could increase the incidence of rearrangements involving the MDR1 gene. Once rearrangement of the gene is established, and MDR1 is under the control of a constitutively active promoter, the overexpression is irreversible.

In summary, the data of Abolhoda et al. (6) tell us that MDR1 induction may occur before the occurrence of gene rearrangement or other mechanism of fixed overexpression. Acute induction of Pgp may lead to the survival of resistant clones and, in reducing the intracellular accumulation of the drug, may encourage the inception of other mechanisms of drug resistance. The transcriptional activation associated with induction may also facilitate the development of constitutive mechanisms of MDR1 overexpression. Because the sarcomas in the study of Abolhoda et al. (6) expressed low levels of Pgp before exposure to chemotherapy, the lesson is that Pgp may be important, even in so-called nonexpressing tumors. These arguments speak to the importance of adding resistance prevention agents at the beginning of cancer treatment.

Although several difficulties will have to be overcome, resistance prevention studies ought to be the next focus for the development of Pgp antagonists. Difficulties include the need for randomization; the large trial size required for a study in initial therapy in sarcoma, lymphoma, breast cancer, or other chemosensitive tumors; and the design issues raised by the pharmacokinetic interactions resulting from the addition of Pgp antagonists to chemotherapy. By failing to face these challenges, we fail to address a mechanism of drug resistance that not only limits some of our most important conventional chemotherapeutic agents but will undoubtedly limit new agents to come.

**REFERENCES**


Drug Resistance: Still on the Learning Curve

Susan E. Bates


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/5/11/3346

Cited articles
This article cites 20 articles, 13 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/5/11/3346.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/5/11/3346.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.